HOMERIA COLLINA, VENT.

- VAR. MINIATA.

An Investigation into its Pharmacological Action

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INTRODUCTORY.

The subject of this investigation is the Homeria collina, Vent. - var. miniata, a native of South Africa, whose natural order is the Irideae. The plant has been imported into Australia and the specimens which were available for pharamacological investigation, had been sent by Mr. D. McAlpine, Government Botanist, Melbourne, to Sir Thomas R. Fraser who kindly permitted me to use them for the following research. Homeria collina is also described under the name of Moraea collina and is known colloquially in Cape Colony as the "Cape Tulip", the "Tulp" or "Tulp-bloem". It is in the Cape Western Province that the variety miniata is most abundant, while other varieties of Homeria flourish in other parts of the country. In Australia the variety miniata appears to be the only representative of the species. That it is poisonous to cattle has been recognised for many years, and in 1906 an Australian Agricultural Journal contained a brief description of the plant and a note on its poisonous nature, (1). In South Africa too, the "Tulp" is well known to be injurious to stock. Cattle reared in a locality where the plant grows do not eat it, but other cattle passing through such a district are tempted to eat the luxurious/

luxurious green shoots and some of them die within 24 hours of eating these. Mr. McAlpine fed rabbits and cows on the aerial parts of Homeria collina and the resulting symptoms (2) led him to the same conclusion as is expressed in Professor Wallace's "Farming Industries of Cape Colony" page 95, viz:- that the Cape Tulip "contains a violent irritant which . . . . induces violent inflammation of the stomach and bowels and . . . . usually death within 24 hours." How far exact investigation supports this opinion will appear later.

Instances of poisoning in man are not common. The bulbous portion of the plant has been eaten in ignorance of its properties, and in such cases severe nausea occurred in a few hours, rapidly followed by vomiting and prostration. A fatal termination resulted in two out of four cases within twelve hours (3), and post-mortem examination revealed intense inflammation of the stomach and small intestines. It is not recorded that diarrhoea occurred. In another instance (4) six persons partook of the bulbous portion of the plant after it had been roasted in the ashes. About half an hour later they were seized with nausea and vomiting and four of them died within 12 hours. The other two survived. Apparently diarrhoea does not occur in man as a result of eating the/

the underground portion of the plant.

Accompanying the specimens was the following botanical description by Baron von Mueller:- Homeria collina, Vent. - var. miniata. A native of South Africa. Bulb almost spherical, covered closely by a coating of interwoven fibres, between the layers of which numerous minute readily sprouting bulbils are concealed. Whole plant to 3 feet high, but usually much less, variable also in more or less robustness or slenderness, often somewhat branched. Leaves linear to 1½ feet long to 2/3 inch broad, but frequently of much less size, always channeled and gradually much narrowed upwards; grey-green above, dark green beneath, slightly streaked, small bulbils also formed occasionally in the axils of some leaves. Inflorescence fascicularly compound when well developed. Somewhat paniculate, the supporting lowest floral leaf often much elongated, clasping at the base. Bracts comparatively long, much pointed, the outer green, the inner smaller gradually colourless, and very tender. Flower stalks to 2 inches long, though often shorter. Some of the stalklets finally to 1½ inches long, all enclosed in longitudinally convolute bracts. Lobes of the calyx 3 (or exceptionally 2), petal-like lanceolar-ovate, about ¾ inch long, yellowish towards the base, otherwise almost brick/

brick-coloured, or nearly orange-coloured. Petals similar to the calyx-lobes but somewhat narrower, 3 (or exceptionally 2), along with the calyx lobes twisted after flowering, finally deciduous. Stamens 3 (seldom 2), much shorter than the calyx lobes and petals. The three anthers erect, seated on the yellowish narrow staminal tube, about ¼ inch long, yellow, broad-linear, blunt, at the base minutely bi-lobed, bursting marginally. Style filiform, about as long as the stigmas. These as well as the anthers, opposite to the calyx lobes, three (or exceptionally 2) in number; hardly extending beyond the anthers, yellowish, linear-cuneate, with numerous dilated bi-lobed crenulated and ciliolated summit, and with two small tender inner appendages. Ovulary quite connate with the calyx-tube, 3-celled (or seldom 2-celled), cylindric and somewhat angular. Ovules very numerous, fixed along the axis. Fruit dry, trigonous cylindric, dehiscent, many seeded. The flowers are distinctly smaller than those of Homeria collina, their petals and calyx-lobes are more acute and of a lighter red; also less venulated, and the staminal tube is glabrous.

These "bulbs" are, in strict botanical language, corms - in other words, each is a short, thick, fleshy stem covered by numerous scale-leaves and bearing one or more buds at its apex. The supply received from Australia weighed 1,190 grams. They were of different sizes, some being no larger than cherry stones while others were even larger than walnuts. In Plate I, two of the larger corms are depicted. The actual colours of the corms and of the parts visible on dissection have been reproduced.

One hundred of the smallest corms weighed 103.75 grams, and thirty of the largest weighed 103.35 grams. Each of these corms was weighed by itself and its greatest circumference was measured. The average weight of these selected corms was 1.6 grams, and their average circumference was 5.75 c.m.

The next step in the investigation was to discover by which menstruum the most active extract was obtainable. The corms were crushed to a coarse powder and dried in vacuo over strong sulphuric acid till no further loss of weight occurred. 50 grams of the dried powder were extracted with methylated ether and yielded 0.85 grams of an extract, which appeared to possess no toxic action.

The marc was next extracted with ethyl alcohol (90%) and yielded 1.2 grams of extract. 0.05 gram of this extract per kilogram produced only slight effects/

effects in rabbits when administered by subcutaneous injection.

The same marc was thereafter exhausted with alcohol (20%) and yielded 1.719 grams of extract. By subcutaneous injection into rabbits the minimum-lethal dose of this extract was found to be about 0.04 gram per kilogram.

It was evident that a dilute alcohol was a better menstruum than a strong one, but after a few days the percolation in the case of 20% alcohol was very slow, owing to swelling of the marc. It was resolved, therefore, to use alcohol 45% as the medium for extracting the active substances.

A thousand grams of the dried powdered corms were taken and the substances soluble in ether were removed by maceration and percolation with Methylated Ether (S.G.-0.730). This extract weighed 7.9 grams. It was of a dark brown colour and gave a permanent translucent stain to paper. The last traces of ether were now driven from the marc by gentle heat; the marc was allowed to cool and was then macerated with five times its bulk of ethyl alcohol (45%. S. G. -.0.944).

The extract obtained after concentration of the percolate at a temperature below 50°C, and subsequent drying <u>in vacuo</u> over strong sulphuric acid weighed

27/

27 grams, and with it the pharmacological action of Homeria collina was investigated.

This extract was finely powdered and thoroughly mixed. It was then of a yellowish brown colour and very hygroscopic.

Many chemical reagents were applied to portions of this extract and to sections of the corm without any characteristic colour reactions being obtained, with the possible exception of the following reaction of sections of the corm with strong sulphuric acid:-

The scale leaves at once become a very dark brown. Within one minute faint streaks of a carmine colour appear in the reserve stored in the swollen stem, especially at the periphery of the section, and spread inwards slowly. In 1 hour the reserve material is almost entirely of a carmine colour, which afterwards becomes black.

The alcoholic extract made with 45% alcohol is completely soluble in cold water, forming a brown solution. It is only partly soluble in absolute alcohol.

(a) When 2 c.c. of a 1% solution of the alcohol extract is heated with 1 c.c. of Fehling's solution, reduction occurs before the boiling point is reached.

(b) 0.2% of sulphuric acid was added to 2 c.c. of the/

the 1% solution and the whole was left at room temperature (13°C.) for 24 hours. The mixture was then neutralised with sodium bicarbonate; 1 c.c. of Fehling's solution was added and, on heating, reduction occurred below the boiling point. The deposit was not appreciably larger nor was the supernatant fluid paler than in the case of (a).

(c) 0.2% of sulphuric acid was added to 2 c.c. of the 1% solution, and the mixture was maintained at 70°C. for 1¼ hours. Then sodium bicarbonate was added to neutralise the free acid, and thereafter 1 c.c. of Fehling's solution was added. When heat was applied to this mixture a copious reduction occurred below the boiling point. The deposit was appreciably larger and the supernatant fluid paler than was the case in (a) and (b). This points to the presence of a glucoside in the alcoholic extract.

The addition of a 10% solution of hydrochlor - platinic acid in absolute alcohol to the clear solution which is formed after agitating 10 c.c. of absolute alcohol with 0.2 gram of the alcoholic extract is at once followed by the formation of abundant minute yellowish crystals of microscopic size. This would appear to point to the presence of an alkaloidal substance in the extract.

PHARMACOLOGICAL ACTION.

Ethereal Extract:- The occurrence of diarrhoea in those animals which have eaten the aerial part of Homeria collina, made it important to investigate the action of those substances in the corms which are soluble in ether.

a. Frogs.

The administration by the mouth of doses up to 2 grams per Kilogram produced no symptoms in frogs. With the largest doses there was evidence of a large proportion having passed through the alimentary canal unchanged.

b. Rabbits.

Administration by the mouth:- With doses of the ether extract up to 1 gram per Kilogram there was no evidence of any action on the alimentary tract within 48 hours, nor did any other symptoms appear.

Administration by subcutaneous injection:- the subcutaneous injection of quantities of ether extract up to 0.1 gram per Kilogram did not result in the manifestation of any symptoms within 24 hours. The difficulty of dissolving or suspending the extract prevented larger doses being given.

It is apparent that those parts of the corms which/

which are soluble in ether have little or no pharmacological action.

Alcoholic Extract free from oil and other substances soluble in ether:-

A. LETHALITY or TOXIC POWER.

The experiments made with this extract on frogs, rabbits and cats have been arranged in tables (I, II, III) to show the minimum quantity capable of producing death when administered by subcutaneous injection. In order to avoid, as far as possible, errors due to differences in the amount of stomach contents in warm-blooded animals (rabbits and cats) the weight was always taken 18 hours after the last reception of food.

Table I. Minimum-lethal Dose of Extract for Frogs.

No. of Expt.	Weight of animal in grams.	Actual dose in grams.	Dose per kilogram in grams.	Result.
1	25	0.00125	0.05	Recovery, distinct effects.
2	35	0.0035	0.1	Recovery, distinct effects.
3	30	0.0042	0.14	Recovery, pro - found effects.
4	25	0.004	0.16	Death in 6 hours.
5	45	0.009	0.2	Death within $3\frac{1}{2}$ hours.
6	40	0.02	0.5	Death in $1\frac{1}{4}$ hours.

Table II. Minimum-lethal Dose of Extract for Rabbits.

No. of Expt.	Weight of animal in grams.	Actual Dose in grams.	Dose per Kilogram in grams.	Result.
7	1900	0.038	0.02	Recovery, distinct effects.
8	2500	0.075	0.03	Recovery, profound effects.
9	2100	0.0735	0.035	Recovery profound effects.
10	2350	0.094	0.04	Death in 2 hours.
11	1930	0.0965	0.05	Death in 2 hours.

Table III. Minimum-lethal dose of Extract for Cats.

No. of Expt.	Weight of animal in grams.	Actual dose in grams.	Dose per Kilogram in grams.	Result.
12	1950	0.039	0.02	Recovery, slight effects.
13	3770	0.1131	0.03	Recovery, distinct effects.
14	2950	0.10325	0.035	Recovery, distinct effects.
15	2300	0.092	0.04	Death in 3 hours.
16	3,000	0.15	0.05	Death in 1 hour 10 minutes.

These tables show that, when the Alcoholic Extract is injected into the subcutaneous tissues, the minimum-lethal dose per Kilogram is in frogs about 0.16 gram, and in rabbits and cats about 0.04 gram.

Frogs/

Frogs (Rana esculenta and temporaria) are, therefore, four times as resistant to this extract as cats and rabbits. Rats which had received by subcutaneous injection 50 times the minimum-lethal dose for rabbits and cats (or 2 grams per Kilogram) manifested slight effects only. In rabbits the intravenous minimum-lethal dose is about 0.035 gram per Kilogram.

B. GENERAL EFFECTS.

A detailed account of several of the experiments in the tables is given below to illustrate the symptoms produced by large non-lethal and by lethal doses, administered subcutaneously.

a. Experiments on cold-blooded animals—Frogs.

Experiment II. A male frog (Rana temporaria), weighing 35 grams, received by subcutaneous injection into the dorsal lymph sac 0.0035 gram of extract dissolved in Ringer's solution. (= 0.1 gram per Kilogram, or $^{5}/_{8}$ of the minimum-lethal dose). Before the injection the throat and flank respirations were regular and at the rate of 30 per 10 seconds, the nose reflex, conjunctival reflex and sacral reflex were acute, the attitude of the frog was normal and the animal jumped well. In 17 minutes after the injection the throat and flank respirations were 26 per 10 seconds, regular in time/

time and deeper; a distinct twitch of the flanks accompanied every fifteenth respiration (or thereabouts); the frog's attitude was unaltered. In 30 minutes after the injection the respirations were at the rate of 22 per 10 seconds and their characters were as last noted; the nose reflex and the sacral reflex were duller, the conjunctival reflex was acute; the frog jumped well, would not remain on its back and its attitude was normal. In 37 minutes after the injection the pupils were more dilated than they had been before, and the frog was restless. In 40 minutes after the injection the throat respirations were at the rate of 21 per 10 seconds and were gulping in character, the flank respirations were irregular in time and feeble, the thorax and abdomen were raised up and the anterior extremities were abducted and semi-extended, the frog was more restless. In 57 minutes after injection the attitude was as last described, the frog was not so restless and its movements were sluggish when it was disturbed. In 1 hour 4 minutes after the injection the throat respirations were 19 per 10 seconds and gulping in character, the flank respirations were half that rate and feeble, the nose reflex and the sacral reflex were dull, and the frog was very restless. In 1 hour/

hour 55 minutes after the injection the throat respirations were 22 per 10 seconds and deep, the flank respirations were 16 per 10 seconds, the reflexes and the attitude were as last noted. In 3 hours 20 minutes after the injection the throat and flank respirations were 20 per 10 seconds and regular in time, the nose reflex and conjunctival reflex were acute; when the point of a blunt needle was placed lightly on the skin just above the upper end of the urostyle one or other posterior extremity was used rapidly to brush it off; the frog jumped well and would remain on its back after a short struggle but recovered the prone position rapidly when the abdomen was stroked; the cardiac impact was very distinct over a large area and at the rate of 10 per second, it was seen to be a double impact.

In 3 hours 30 minutes after the injection the pupils were very small; when the blunt needle was lightly applied (without stroking) to the skin immediately above the urostyle either a pelvic extremity was used to brush it off or the frog extended and adducted its anterior extremities fully and arched its back, croaking meantime and usually ending by falling over on to one side.

In 4 hours 25 minutes after the injection reflex movements occurred as at 3 hours 30 minutes; the frog/

frog remained on its back quietly for one minute and then recovered the prone position spontaneously; the cardiac impacts were very distinct and at the rate of 9 per 10 seconds.

In 15 hours after the injection the throat and flank respirations were regular and at the rate of 24 per 10 seconds, the throat movements were very large and those of the flanks small; the nose reflex was dull, the conjunctival reflex was acute and the sacral reflex was very acute; the application of the blunt needle to the skin just above the urostyle resulted in the same exaggerated movements as at 3 hours 30 minutes, otherwise the frog was not restless but lay with limbs flexed and thorax and abdomen on the tray; it jumped well and would not remain on its back. In 40 hours after the injection the animal's condition and reflexes were as at 15 hours after injection.

In 62 hours after the injection the respirations (throat and flank) were 24 per 10 seconds and regular; the nose and conjunctival reflexes were acute; the sacral reflex was very acute; when the point of a blunt needle was kept applied to the skin just above the urostyle several twitches of the flanks occurred, then the frog croaked loudly and used a pelvic extremity to remove the irritant; when undisturbed the/

the animal was inactive, but jumped very actively when disturbed; it remained on its back quietly but recovered rapidly when the abdomen was stroked; the cardiac impacts were 6 per 10 seconds and seen over a large area; the frog now weighed 38 grams. 5 days after the injection the throat and flank respirations were 24 per 10 seconds and regular in time; the reflex movements were as at 62 hours, the frog jumped well; it would remain on its back for a few seconds only; the cardiac impacts were 9 per 10 seconds.

6 days after the injection the frog would not remain on its back and used its foot to remove the needle point only when the point of the needle was rubbed heavily upon the skin just above the top of the urostyle.

The most striking effects which followed the injection in this experiment are:- early slowing and deepening of the throat respirations, slowing and irregularity of the flank respirations; an increase of the force of the cardiac impacts against the abdominal wall and of the area over which they are visible (as compared with normal frogs) and the appearance of a double impact (auricular and ventricular); a well-marked exaggeration of reflex movements, which lasted for several days.

Experiment IV./

Experiment IV. (curtailed) A male frog (Rana temporaria) weighing 25 grams, received by subcutaneous injection into the dorsal lymph sac 0.004 gram of extract dissolved in Ringer's solution (= 0.16 gram per Kilogram or the minimum-lethal dose). Before the injection the throat and flank respirations were 17 per 10 seconds and regular in time; the nose reflex, conjunctival reflex and sacral reflex were acute; the posture was normal and the frog jumped well. During three hours after the injection the respirations (throat and flank) became slowed and irregular and the three reflexes were more acute.

In 3 hours 30 minutes after the injection the throat and flank respirations were 6 per 10 seconds, irregular in time and feeble; the three reflexes were much more acute; when the skin over the sacrum was lightly stroked with a blunt needle, both pelvic extremities were rapidly flexed and swept across the back to remove the irritant; when the blunt needle-point was gently placed on the skin of a thigh, that pelvic extremity was kicked out vigorously; the frog would remain on its back quietly when placed there, and meantime all the limbs were flexed strongly and the muscles of the thorax and abdomen were tense; the cardiac impacts were/

were not visible; the frog jumped well.

In 5 hours 55 minutes after the injection there was no visible respiratory or cardiac movement; the conjunctival reflex was sluggish and the sacral reflex feeble; the frog lay flaccidly on its abdomen and thorax with the head somewhat retracted; but slight resistance was offered to passive extension of one of the posterior extremities; when the limb was passively extended it was not flexed again unless the toes were pinched strongly and then flexion was slow and feeble.

In 6 hours after the injection the frog was decapitated and the thorax was opened; the heart was motionless, the auricles were large and dark blue in colour, the ventricle was small, well contracted and only moderately pale; local mechanical stimulation of the auricles caused them to contract feebly and superficially but the ventricle was unaffected; when a blunt needle-point was lightly drawn across the ventricle its course was marked by a permanent white line due to local contraction, and, after several such stimuli, the whole ventricle became quite pale and firmly contracted; a section of the heart muscle gave a well-marked acid reaction with litmus paper; a section of the thigh muscles was faintly acid to litmus paper.

In/

In 6 hours 10 minutes after the injection (and 10 minutes after decapitation) the sciatic nerve and the gastrocnemius muscle of the left side were exposed and when tested by means of a Du Bois Reymond's apparatus, using Neef's hammer and a 4-volt accumulator, direct stimulation of the sciatic nerve with the secondary coil at 330 m.m. caused a contraction of the gastrocnemius, and the muscle itself responded to direct stimulation with the secondary coil at 180 m.m.

In 6 hours 30 minutes after the injection (and 30 minutes after decapitation) the response of the gastrocnemius to electrical stimulation of the sciatic nerve and of the muscle directly was the same as at 6 hours 10 minutes.

When the oesophagus was divided longitudinally, it was found to contain the invaginated stomach, part of which was visible inside the mouth. After the injection in this experiment the respiratory movements of the throat and flanks were markedly slowed and became irregular in time and finally disappeared; the reflex movements became greatly exaggerated and later they were greatly diminished. Before the reflexes had disappeared completely, it was found that the heart was arrested with/

with the auricles engorged and the ventricle well contracted and that all the heart's chambers were contractile; a section of the ventricle was acid to litmus paper, as was also a section of skeletal muscle. 30 minutes after decapitation the gastrocnemius responded to electrical stimulation of its sciatic nerve with the secondary coil at 330 m.m. and to direct stimulation with the coil at 180 m.m. There was evidence of vomiting.

Experiment VI. A male frog (Rana temporaria), weighing 40 grams, received by subcutaneous injection into the dorsal lymph sac 0.02 gram of extract dissolved in Ringer's solution (= 0.5 gram per Kilogram, or about three times the minimum-lethal dose). Before the injection the throat and flank respirations were 25 per 10 seconds and regular in time; the nose reflex, conjunctival reflex and sacral reflex were acute, the posture was normal and the frog jumped well. In 10 minutes after the injection the throat and flank respirations were 23 per 10 seconds and regular in time; the attitude of the frog was unchanged. In 20 minutes after the injection the throat respirations were 20 per 10 seconds and regular, the flank respirations were about 20 per 10 seconds, very irregular in time and extent/

extent; the three reflexes were acute and the posture normal. In 30 minutes after the injection the throat respirations were 15 per 10 seconds and slightly irregular in time, the flank respirations were feeble and very irregular in time. In 42 minutes after the injection the throat and flank respirations were 3 per 10 seconds and sometimes no visible respiratory movements occurred in 10 seconds; the conjunctival reflex was sluggish, and the nose reflex and sacral reflex were absent; the pupils were very small, the head was somewhat retracted and the mouth often gaped; the limbs were flexed.

In 47 minutes after the injection the mouth was half opened and remained so for more than a minute; meantime the pupils became very small; then the mouth gaped widely, the abdominal muscles contracted powerfully, the floor of the mouth was seen to be pushed upwards and the upper part of the oesophagus prolapsed temporarily; after an interval of 10 seconds this was repeated, and the mouth remained half open.

In 50 minutes after the injection the conjunctival reflex was present and sluggish; the sacral and nose reflexes were absent.

In 51 minutes after the injection the mouth was still open; it gaped widely so that the two alveolar/

alveolar margins formed almost a straight line, the gloths opened wide and then closed; then a violent contraction of the abdominal muscles occurred causing a temporary slight prolapse of the oesophagus . These retching movements were repeated at intervals of from a half to one minute during the next four minutes. By this time the respiratory movements were entirely absent.

In 55 minutes after the injection the frog remained on its back when placed there; the anterior abdominal wall showed ridges corresponding to contracted muscles and converging from the thorax to the pubic symphysis; no cardiac impacts were visible.

In 57 minutes after the injection the conjunctival reflex could still be elicited; the pelvic extremities were flexed so firmly that they could not be extended by pulling on them with considerable force; this unsuccessful attempt to extend them passively was at once followed by violent retching.

In 1 hour after the injection slight gaping movements and feeble expulsive movements were occurring. Half a minute later these were repeated, and now the pupils were less contracted.

In 1 hour 2 minutes after the injection one posterior extremity was passively extended by using considerable/

considerable force, a general struggle resulted but there was no retching; the toes were then pinched and the frog jumped away and retching occurred, the tongue was protruded and fibrillary twitches were seen to be present in it.

In 1 hour 3 minutes after the injection the pupils were semi-dilated and the conjunctival reflex was absent; the frog lay prone and flaccid, and made no effort to resume the prone position when placed on its back; there were no visible cardiac impacts; pinching the toes caused a general struggle but there were no expulsive movements; the mouth was almost closed.

In 1 hour 32 minutes after the injection the frog was decapitated and the thorax was opened. The heart was motionless, the auricles were engorged and the ventricle was small and very pale with the exception of a single small dark area on the anterior wall; none of the chambers responded to local mechanical stimulation; a section of the ventricle was faintly acid to litmus paper. The entire stomach as far as its pyloric end was found everted inside the mouth; the intestines were slightly congested.

In 1 hour 36 minutes after the injection a gastrocnemius muscle responded to stimulation of its sciatic nerve with the secondary coil at 80 m.m., and to direct stimulation at 70 m.m. A section of the muscle gave a feebly/

feebly acid reaction with litmus paper.

After the administration of the extract in this experiment the respirations rapidly became markedly slower and irregular in time; the skin reflexes disappeared rapidly, the conjunctival reflex became very sluggish and finally disappeared. Violent retching occurred repeatedly and considerable rigidity of the skeletal muscles was manifested; when these effects had passed off, the heart was found to be motionless with the auricles engorged, the ventricle pale and firmly contracted and all the chambers inexcitable. The gastrocnemii still responded to direct electrical stimulation and to electrical excitation of their nerves. Sections of the heart and thigh muscles were acid to litmus paper.

b. Experiments on warm-blooded animals.

1. Rabbits.

Experiment VIII. A male rabbit, weighing 2500 grams, received by subcutaneous injection into the left flank 0.075 grams of extract dissolved in Ringer's solution (= 0.03 gram per Kilogram, or $\frac{3}{4}$ of the minimum-lethal dose). Before the injection the respirations were at the rate of 13 per 10 seconds, regular and moderately deep, the cardiac impacts were 30 per 10 seconds, regular and distinct, the transverse/

transverse diameter of the left pupil was 6 m.m., the conjunctival reflex was acute and the rabbit's attitude was normal.

In 5 minutes after the injection the rabbit was a little restless. In 10 minutes after the injection the respirations were regular and at the rate of 10 per 10 seconds, the cardiac impacts were at the rate of 30 per 10 seconds, regular and distinct, and the rabbit was more restless.

In 30 minutes after the injection the respirations were 11 and the cardiac impacts 28 per 10 seconds and regular, the conjunctival reflex was acute.

In 45 minutes after the injection the respirations were 17 and the cardiac impacts 24 per 10 seconds, both being regular; the conjunctival reflex was acute and fine tremors were present in the muscles of the shoulders and upper arms.

In 55 minutes after the injection the respirations were 17 per 10 seconds, regular and moderately deep; the cardiac impacts were 30 per 10 seconds, regular and shock-like in character; the conjunctival reflex was acute and the transverse diameter of the left pupil was 6 m.m.

In 1 hour 5 minutes after the injection only 11 cardiac impacts could be felt in 10 seconds and the respirations/

respirations were slow and inspiration laboured during three minutes.

In 1 hour 10 minutes after the injection the respirations were 20 per 10 seconds and deeper, the cardiac impacts were 24 per 10 seconds and slightly irregular in time; and the fore-feet frequently slipped forward.

In 1 hour 20 minutes after the injection the respirations were at the rate of 27 per 10 seconds regular, and as when running; the cardiac impacts were 11 per 10 seconds, irregular in time and more diffuse; the back was less arched and the thorax and abdomen rested flaccidly on the tray.

In 1 hour 25 minutes after the injection the respirations were 22 and the cardiac impacts 12 per 10 seconds and their characters were as at 1 hour 20 minutes.

In 1 hour 30 minutes after the injection the respirations were 26 and the cardiac impacts 28 per 10 seconds and their characters were as at 1 hour 20 minutes; the right haunch now rested on the tray.

In 1 hour 35 minutes after the injection the respirations were 28 per 10 seconds and shallow; the cardiac impacts were 35 per 10 seconds, irregular in time and feeble; the conjunctival reflex was acute, the transverse diameter of the left pupil was 7 m.m., and/

and each respiration was accompanied by an abrupt movement of the head.

In 1 hour 50 minutes after the injection the respirations were 20 and the cardiac impacts 32 per 10 seconds; the head and ears were half erect, the back was less arched, the anterior extremities were widely abducted and a fine tremor was palpable in the muscles of the thorax; when placed on the floor, the rabbit moved about slowly.

In 2 hours after the injection the respirations were 20 per 10 seconds, the cardiac impacts were 34 per 10 seconds, regular and feeble; the conjunctival reflex was acute, the transverse diameter of the left pupil was 6 m.m., and the rabbit lay flaccidly extended on thorax and abdomen.

In 2 hours 30 minutes after the injection the respirations were 19 per 10 seconds, regular and s shallow; the cardiac impacts were 34 per 10 seconds, irregular in time and feeble; the rabbit lay flaccidly on thorax and abdomen and fine tremors were present in the muscles of the neck and shoulders.

In 2 hours 45 minutes after the injection the respirations were 19 and the cardiac impacts 35 per 10 seconds, the latter were feeble; the rabbit's haunches frequently fell to one or other side and then the normal position was recovered abruptly: the head/

head moved abruptly with the respirations.

In 3 hours 5 minutes after the injection the respirations were 14 and the cardiac impacts 32 per 10 seconds; the latter were feeble and regular in time and force.

In 3 hours 30 minutes the respirations were 12 per 10 seconds and there was a visible coarse tremor of the abdominal walls at the end of expiration; the cardiac impacts appeared to be at the rate of 27 per 10 seconds, and were very feeble and irregular in time.

In 3 hours 50 minutes after the injection the respirations were 9 per 10 seconds and the tremor at the end of expiration was still present; the cardiac impacts were 34 per 10 seconds, regular and feeble; the conjunctival reflex was acute and the transverse diameter of the left pupil was 6 m.m., the rabbit lay flaccidly with thorax and abdomen on the tray, the haunches often fell over to one side, and voluntary movements of the head were accompanied by a clonic spasm.

In 4 hours 10 minutes after the injection the respirations were 14 and the cardiac impacts 34 per 10 seconds, the latter were regular in time and feeble.

In 4 hours 25 minutes the respirations were 17 per 10 seconds and regular, the cardiac impacts were

36/

36 per 10 seconds, regular and less abrupt in character; the rabbit was lethargic.

In 5 hours 25 minutes the respirations were 16 per 10 seconds and there was no visible tremor, the cardiac impacts were 40 per 10 seconds and regular; the muscular weakness was less marked.

In 6 hours after the injection the respirations were 21 per 10 seconds, regular and fairly deep; the cardiac impacts were 35 per 10 seconds and regular; the back was well arched and the rabbit was alert and active.

In 24 hours after the injection the rabbit seemed quite recovered.

Following the administration of the extract in this experiment the respirations became at first only more rapid, later they were also more feeble and irregular in time; periods of inspiratory dyspnoea occurred. The cardiac impacts became somewhat slower and more powerful; later they were irregular in time, and feeble and irregular in character. The pupil and the conjunctival reflex were unaffected. Fine tremors occurred in the skeletal muscles and great muscular weakness developed. Finally there was complete recovery within 24 hours.

Experiment X. A male rabbit, weighing 2350 grams, received by subcutaneous injection into the left flank 0.094 gram of extract in Ringer's solution (= 0.04 gram per Kilogram or the minimum-lethal dose). Before the injection the respirations were 10 per 10 seconds, regular and deep; the cardiac impacts were 35 per 10 seconds, regular and easily felt; the transverse diameter of the left pupil was 6 m.m., the conjunctival reflex was acute, the rectal temperature was 38.5°C., and the posture was normal.

In 30 minutes after the injection the respirations were 13 per 10 seconds and slightly irregular in time, the cardiac impacts were 44 per 10 seconds, regular and easily felt, the conjunctival reflex was acute and the posture was normal.

In 40 minutes after the injection the respirations were 19 per 10 seconds and regular in time.

In 45 minutes after the injection the respirations were 20 per 10 seconds and irregular in time; the cardiac impacts were 42 per 10 seconds and irregular in force.

In 1 hour after the injection the respirations were 18 per 10 seconds and irregular in time; periods of inspiratory dyspnoea occurred during which the neck was craned forward, and the respirations were slow, inspiration being difficult and often accompanied by a sucking sound apparently produced in the glottis/

glottis; chewing movements of the jaws occurred from time to time.

In 1 hour 5 minutes after the injection the respirations were 17 per 10 seconds and irregular in time; the cardiac impacts were 52 per 10 seconds, irregular in time and feeble; the transverse diameter of the left pupil was 6 m.m., and the rectal temperature was 37.5°C.

In 1 hour 10 minutes after the injection the rate of the cardiac impacts appeared to be between 40 and 50 per 10 seconds, the impacts were very irregular in time and force; the rabbit sat with head and ears quite erect, and the back was well arched.

In 1 hour 17 minutes after the injection the respirations were 17 per 10 seconds and irregular in time; the cardiac impacts were 48 per 10 seconds, irregular in time and tapping in character; the head moved abruptly with the respirations, the rabbit was restless and passed a considerable amount of urine.

In 1 hour 20 minutes after the injection the animal sat well back on its haunches, the anterior extremities fully extended and closely adducted and the head well raised; the cardiac impacts were very irregular in time and force and the rabbit was restless.

In 1 hour 25 minutes after the injection the animal was able to maintain this last attitude for a few/

few seconds only; it repeatedly sank down slowly and then raised itself.

In 1 hour 31 minutes after the injection the palpable cardiac impacts were 29 per 10 seconds, very irregular in time and feeble, the conjunctival reflex was acute and the rabbit lay flaccidly on its thorax and abdomen with the head slowly sinking downwards.

In 1 hour 35 minutes after the injection the respirations were 19 per 10 seconds, regular and shallow, the rabbit was sitting erect, but slowly sank down; the rectal temperature was 37.5° C.

In 1 hour 42 minutes after the injection the muzzle rested on the tray and the back was much less arched than normally.

In 1 hour 46 minutes after the injection the respirations were 12 per 10 seconds, inspiration being very abrupt and shallow; the rabbit repeatedly sat erect suddenly and slowly sank down again.

In 1 hour 50 minutes after the injection several general clonic convulsions occurred in rapid succession; during the next two minutes the cardiac impact could be felt though feeble and irregular in time and force; the pupils were more contracted, and the conjunctival reflex was acute.

In 1 hour 55 minutes after the injection the pupils which had been contracting dilated suddenly and/

and the conjunctival reflex was absent; the rabbit gave a single gasping expiration and was dead.

In 2 hours after the injection (and 5 minutes after death) the pupil was contracting rapidly.

In 2 hours 5 minutes after the injection (and 10 minutes after death) the thorax was opened; the right ventricle was contracting feebly and spontaneously, the left ventricle was motionless, inexcitable and moderately small and pale; the right auricle was large and dark coloured, the left auricle was small and bright red; a section of the ventricular muscle gave an acid reaction with litmus paper as did a section of the thigh muscles. The blood was fluid and dark in colour. The abdominal and thoracic organs were healthy. Stimulation of a sciatic nerve with the secondary coil at 120 m.m. caused a contraction of its muscles; the muscles themselves responded to direct stimulation at 150 m.m., A localised contraction of the diaphragm was obtained on stimulating a phrenic nerve at 60 m.m., and on direct stimulation of the muscle at 40 m.m.

After the injection in this experiment the respirations were accelerated and later became irregular in time, periods of inspiratory dyspnoea occurred; the cardiac impacts were increased in rate and soon became irregular in rate and in force; after death the left ventricle was found to be motionless, contracted/

contracted and inexcitable, and a section of its muscle was acid in reaction; skeletal muscle, which was also acid, was excitable as were the phrenic and sciatic nerves.

In the course of the experiment muscular weakness developed. The rectal temperature and the condition of the pupil and of the conjunctival reflex were unaffected until shortly before death.

2. Cats.

Experiment XV. A female cat, weighing 2300 grams, received by subcutaneous injection into the right flank 0.092 gram of extract dissolved in Ringer's solution (= 0.04 gram per Kilogram, or the minimum-lethal dose). Before the injection the respirations were 6 per 10 seconds, regular and gentle; inspiration and expiration were of equal length; the cardiac impacts were 36 per 10 seconds, regular and easily felt; the conjunctival reflex was acute and the posture was normal.

In 30 minutes after the injection the respirations were 6 per 10 seconds, the cardiac impacts were 34 per 10 seconds, and both were unchanged in character; the attitude was normal.

In 43 minutes after the injection the respirations/

tions were 6 per 10 seconds, the conjunctival reflex was acute, the animal was restless, licked its lips and it growled and struggled when handled.

In 48 minutes after the injection the cat continued to lick its lips. One minute later it retched and vomited. Half a minute later it vomited again.

In 51 minutes after the injection the respirations were 7 per 10 seconds, regular in time, and expiration was almost twice as long as inspiration; the animal snarled when efforts were made to feel the cardiac impacts.

In 57 minutes after the injection the cat retched and vomited; this was repeated in half a minute.

In 63 minutes after the injection the respirations were 6 per 10 seconds and regular in time, expiration was twice as long as inspiration; the cat was lying quietly on thorax and abdomen but moved uneasily from time to time.

In 1 hour 5 minutes after the injection the animal moved its lips and licked them; half a minute later it retched and vomited twice.

In 1 hour 9 minutes after the injection the respirations were 7 per 10 seconds and regular in time, expiration was more prolonged than inspiration which was abrupt; the cat changed its position frequently.

In/

In 1 hour 12 minutes after the injection the cat licked its lips, retched and vomited twice.

In 1 hour 19 minutes after the injection the animal lay quietly extended on its left side. Three minutes later it licked its lips, retched and vomited.

In 1 hour 30 minutes after the injection the respirations were 5 per 10 seconds and regular in time, expiration was three times as long as inspiration.

In 1 hour 35 minutes after the injection the respirations were 4 per 10 seconds and regular in time; expiration was about twice as long as inspiration which was less abrupt; the animal lay quietly on its left side with its eyes half closed.

In 1 hour 40 minutes after the injection the cardiac impacts were 32 per 10 seconds, regular and not powerful; no muscular tremors were seen. Six minutes later the cat rose, moved uneasily, retched violently and vomited.

In 1 hour 50 minutes after the injection the pupils became widely dilated, and the respirations were very rapid; then violent clonic contractions of the neck muscles jerked the head backwards; thereafter the cat extended itself quietly on abdomen and thorax and the pupil contracted.

In 1 hour 58 minutes the respirations were 7 per 10 seconds, quiet and regular in time; expiration and inspiration were of equal length, and the animal was lethargic.

In/

In 2 hours 15 minutes after the injection the respirations were 5 per 10 seconds, regular and gentle; the cardiac impacts were 24 per 10 seconds, regular in time and easily felt, though not more easily than before injection; the pupils were contracted and the cat was lethargic.

In 2 hours 35 minutes after the injection the respirations were 5 per 10 seconds and regular in time; inspiration was gentle and rapid, expiration was more forced and prolonged and began with a sudden contraction of the abdominal muscles. The cardiac impacts were 28 per 10 seconds, regular in time and easily felt; the animal was lethargic and could be roused only momentarily.

In 2 hours 52 minutes after the injection a general tonic convulsion occurred after which the cat lay still for 30 seconds and then moved about a little. This was repeated three and four minutes later.

In 2 hours 57 minutes the respirations were occasional and gasping, with prolonged expiration; the pupil was widely dilated, the conjunctival reflex almost absent, and the cat lay extended on its side.

In 3 hours after the injection the pupil was widely dilated, the conjunctival reflex was absent, and the cat was dead.

In 3 hours 2 minutes after the injection (and 2 minutes after death) the thorax and abdomen were opened; the heart was motionless with the exception of/

of the left auricle which was contracting feebly and superficially; the right auricle was dark and dilated, the left auricle was moderately large and of a bright red colour; the right ventricle was moderately contracted while the left ventricle was smaller than the right owing to its being more contracted; all the heart's chambers responded to direct mechanical stimulation and a section of the ventricles was feebly acid in reaction. A section of the thigh muscles was also acid to litmus paper.

In 3 hours 4 minutes after the injection (and 4 minutes after death) the stomach was small, pale and firm to the touch; its cavity was empty and the rugae were very distinct; the duodenum and the jejunum were pale, firm and empty, the ileum was less so; active peristaltic movements were occurring; the caecum and upper part of the large intestine contained fluid faecal matter; the lower part of the large intestine and the rectum were pale, rigid and empty; the abdominal and thoracic organs were healthy.

After the administration of the extract in this experiment the respirations gradually became slower, expiration was prolonged and inspiration was at times very rapid; the cardiac impacts were slowed and soon after death the auricles were found to be fairly large while the ventricles were small; all the chambers were/

were excitable; the left ventricle especially was well contracted and a section of it was acid in reaction as were sections of skeletal muscle; repeated attacks of violent retching and vomiting occurred throughout the experiment, and after death the stomach and the small intestine were small, pale and empty; in the earlier part of the experiment the animal was restless but later it became lethargic; general convulsions occurred just before death.

Administration by the Mouth.

Experiments were performed in which the alcoholic extract was administered by the mouth to frogs, rabbits and cats. It was found that the resulting symptoms were identical with those just described but that the dose per Kilogram necessary to produce them was four to six times greater than that by subcutaneous injection. The appearance of the symptoms (including vomiting) was somewhat retarded and no evidence of gastro-intestinal irritation was obtained beyond that which has already been described in detail. (Experiments VI and XV).

Effect of Heat. In order to ascertain whether prolonged heating destroyed the activity of the extract, a rabbit, weighing 2,200 grams, was given by subcutaneous injection into the left flank 0.22 gram of extract dissolved in Ringer's solution (= 0.1 gram per Kilogram or $2\frac{1}{2}$ times the subcutaneous minimum-lethal dose). Before being injected, the dose was kept at a temperature of 100° C. for 30 minutes and then allowed to cool. The animal developed the usual symptoms produced by Homeria collina, and died in 1 hour 35 minutes after the injection. Immediate post-mortem examination revealed the same conditions of the heart as are present in rabbits after death following the injection of the unheated extract. The activity of the extract, therefore, is not destroyed by heating up to 100° C. for 30 minutes.

Summary of General Effects in Frogs.

When a dose of the extract sufficient to cause death within three hours is administered subcutaneously to a frog the following effects are produced:- Soon after the injection the respiratory movements are slowed and become irregular in time, the flank respirations showing these effects earlier than the throat respirations; respiratory movements of the throat occur when no cardiac impact can be detected. The spinal reflex is exaggerated (but this is more easily demonstrated with large sublethal or small lethal doses) and repeated violent retching occurs. Accompanying this increase in the reflex movements, symptoms of muscular rigidity develop; at first the head is retracted and later all the limbs are strongly flexed and the muscles of the abdominal wall are powerfully contracted; expulsive movements continue to occur but are more feeble. Gradually the muscular rigidity becomes less marked, but active reflex movements can still be elicited. If, now, the thorax be opened, the heart is usually found to be motionless with the ventricle pale, small and inexcitable; the auricles are large and dark coloured, and they may be motionless or feebly contracting; they may respond to mechanical stimulation, but their movements produce no visible effect upon the ventricle; a section of the ventricular muscle is acid to litmus paper.

The/

The skeletal muscles are contractile and motor nerves are excitable for a short time after death, and the muscles become acid in reaction soon after the heart muscle.

Summary of General Effects in Rabbits.

Soon after a dose of the extract sufficient to cause death within three hours is administered subcutaneously to a rabbit, the respiratory movements become slightly accelerated and they may become irregular in time, the rate and force of the cardiac impacts is increased and the animal is more alert; periods of inspiratory dyspnoea usually occur, accompanied by sounds apparently produced in the glottis, and chewing movements of the jaws may be observed. These symptoms are followed rapidly by irregularity in the force of the cardiac impacts and a further increase in their rate, while the respirations are usually slowed; the rabbit's head is well raised, its back very well arched and its anterior extremities closely adducted and fully extended. Thereafter signs of muscular weakness develop, the head begins to sink forward, at first momentarily only, but later the muzzle rests continuously on the tray and the back loses its convexity. Still later the side of the/

the head rests on the tray and the rabbit falls over on to one side and finally the animal lies quietly extended on one side, breathing rapidly. By this time the cardiac impacts are thumping in character or usually only tapping; they are very irregular in time and in force and soon become almost impalpable. Thereafter general clonic convulsions occur and the pupils, which have previously contracted slightly, now dilate rapidly and the conjunctiva becomes insensitive. If the heart be at once exposed, it may be found to be contracting feebly or to be quite motionless. If motionless, it may still respond feebly to mechanical stimulation; the left ventricle is smaller and paler than the right and a section of the ventricular muscle gives an acid reaction with litmus paper. The skeletal muscles become distinctly acid soon after the heart muscle; immediately after death they are excitable as are the phrenic and sciatic nerves. The rectal temperature, and the condition of the pupil and of the conjunctival reflex are not affected until the animal is moribund. Usually a considerable quantity of urine is passed forcibly in the course of the experiment or at the time of death. In some cases as much as 70 c.c. of urine are found in the bladder after death. The urine presents no abnormal appearances (as of blood); when/

when the abdomen is opened, intestinal peristalsis may be active, but there are no symptoms of gastro-intestinal irritation during life. After death the blood is fluid and clots readily. No evidence of intravascular clotting is found.

Summary of General Effects in Cats.

When a dose sufficient to cause death within three hours is administered subcutaneously to a cat, the train of symptoms is almost the same as in rabbits. The condition of the heart after death is similar and the reaction of the heart and skeletal muscles to litmus paper is acid. Soon after the injection restlessness is evinced, but later the animal becomes lethargic. Slowing of expiration is marked and inspiration becomes rapid. Repeated attacks of violent retching and vomiting occur (with sublethal as with lethal doses) and make it difficult to observe the effects on the respiration and the circulation. Retching begins soon after the injection and more quickly after subcutaneous injection than after administration by the mouth. In fatal cases it continues almost without interruption, until death supervenes, and in non-fatal cases until a short time before the total disappearance of symptoms.

From the foregoing experiments it is evident that the/

the alcoholic extract of Homeria collina acts upon the cardiac and the skeletal muscles and upon parts of the cerebro-spinal nervous system; there may also be a direct action upon respiration.

C. Action on the Cerebro-spinal Nervous System.

a. Brain and Spinal Cord.

Symptoms such as restlessness and lethargy which occurred in the course of experiments to ascertain the general effects of Homeria collina, are doubtless secondary to the striking effects of the extract on the heart. The vomiting which was noted with large lethal doses in frogs and with lethal and large sub-lethal doses in cats may be due, at least in part, to a central action; although vomiting did not begin for 25 minutes after the subcutaneous administration of the extract, it did not occur until more than an hour after the extract had been given by the mouth.

The well-marked increase in the skin reflexes made it probable that there was a direct action on the spinal cord and experiments were performed to ascertain such an action. In these experiments frogs were used; the brain was destroyed in front of a line joining the posterior margins of the eyelids, and thereafter the common iliac, the femoral and the epigastrico-vesical (Ecker) arteries of one side were ligatured. The consequent arrest of the circulation in the posterior extremity of that side was ascertained by examination of the web under the microscope. For electrical/

electrical stimulation a Du Bois Reymond's apparatus was employed with Neef's hammer and a 4-volt accumulator. With the secondary coil at 130 m.m., the current was just felt on placing the electrodes on the tip of the tongue. It was found necessary to give large lethal doses of the extract subcutaneously in order to obtain definite effects on the nervous system in a short time.

Experiment XVI. Half an hour before the injection of 0.0275 gram of extract subcutaneously into the right flank of a male frog (Rana temporaria), weighing 55 grams (= 0.5 gram per Kilogram or about three times the minimum-lethal dose), the vessels supplying the left posterior extremity were ligatured and both sciatic nerves were exposed. After this procedure the attitude of the frog remained normal, and the frog moved slowly away when the uncharged electrodes were placed lightly on either web or on the dorsum just above the urostyle.

During 30 minutes after the injection there was no change in the posture of the animal nor in the reflexes.

In 45 minutes after the injection the lightest touch with the uncharged electrodes anywhere on the skin of the dorsum below the level of the anterior extremities/

extremities and on either side of the middle line caused active efforts to remove the irritant with the pelvic extremity of that side; the application of the uncharged electrodes to the skin of either posterior extremity resulted in that extremity being jerked away rapidly; the frog sat erect and would not remain on its back when placed thereon.

In 55 minutes after the injection the same conditions persisted as noted at 45 minutes.

In 70 minutes after the injection the frog was lying on thorax and abdomen with the anterior extremities flexed loosely; the application of the uncharged electrodes to either web caused the posterior extremity of the same side to be drawn away at once, and when the skin of the dorsum was lightly touched by the uncharged electrodes the frog jumped away.

In 78 minutes after the injection the cardiac impacts on the abdominal wall were visible; application of the uncharged electrodes to either web caused the pelvic extremity of the same side to be withdrawn smartly; merely touching the skin of the legs and dorsum did not produce a reflex but lightly stroking the skin of either leg or of the dorsum caused the frog to jump away.

In 1 hour 28 minutes after the injection the thorax/

thorax was opened; the heart was found to be arrested with the ventricle firmly contracted.

In 1 hour 33 minutes after the injection (and 5 minutes after the arrest of the heart had been ascertained) the right (unprotected) leg was withdrawn on stimulation of its web with the secondary coil at 100 m.m., and the left (protected) leg was withdrawn on stimulation of its web at 120 m.m., a crossed reflex was obtained on stimulating the right (unprotected) web at 90 m.m., and the left (protected) web at 100 m.m., both posterior extremities were drawn up slightly and then rapidly and fully extended when the skin over the spinal cord just above the proximal end of the urostyle was stimulated with the secondary coil at 140 m.m.

In 1 hour 48 minutes after the injection (and 20 minutes after the arrest of the heart had been ascertained) the right (unprotected) leg was withdrawn when its web was stimulated with the secondary coil at 100 m.m., the left (protected) leg was withdrawn when its web was stimulated at 110 m.m., and in both cases a crossed reflex occurred; both pelvic extremities were simultaneously and completely extended when the skin over the cord just above the urostyle was stimulated with the secondary coil at 140 m.m.

In/

In 1 hour 57 minutes after the injection (and 29 minutes after the arrest of the heart had been ascertained) the right (unprotected) leg was withdrawn when its web was stimulated with the secondary coil at 90 m.m., and the left (protected) leg was withdrawn when its web was stimulated at 100 m.m.

In 2 hours 10 minutes after the injection (and 42 minutes after the arrest of the heart had been ascertained) no reflex was obtained on stimulation of either web, even with the secondary coil at zero; stimulation of the skin over the spinal cord just above the urostyle at 100 m.m. caused both pelvic extremities to be rapidly and simultaneously extended, and stimulation of either sciatic nerve at 330 m.m. resulted in a contraction of its gastrocnemius.

Although the experiment was continued until 5 hours had elapsed since the time of the injection (and until 3 hours 32 minutes after the arrest of the heart had been ascertained), no further important changes occurred. When observations were discontinued 5 hours after the injection, stimulation of the skin over the cord just above the urostyle with the secondary coil at 80 m.m. caused both pelvic extremities to be rapidly and simultaneously extended, and direct stimulation of either sciatic nerve at 370 m.m. resulted in a contraction of its gastrocnemius.

On section the right (unprotected) gastrocnemius was distinctly acid and the left (protected) gastrocnemius/

nemius was doubtfully acid to litmus paper.

After the administration of the extract in this experiment there was a distinct increase in the spinal reflexes. The exaggerated reflex movements occurred equally in the protected and in the unprotected posterior extremity, and could be elicited by equal stimuli applied to the skin of the protected and of the unprotected limb. The spinal reflex had disappeared in 42 minutes after the arrest of the heart had been ascertained and at that time the heart could not have been arrested for more than 60 minutes. Experiments in which the heart's movements and the blood circulation are stopped by ligaturing the base of the heart show that the reflex function of the spinal cord persists for at least 2⅔ hours after complete arrest of the heart. In experiment XVI, therefore, there is evidence that the extract of Homeria collina exaggerates, and later abolishes, the reflex function of the spinal cord by a direct action on the cord itself.

The skeletal muscles to which the extract had access were distinctly acid when muscles protected from the poison were neutral or very feebly acid in reaction.

b. Sensory Nerves.

In order to investigate the effects of the extract on afferent nerves, large frogs (Rana esculenta) were used. Their brains were destroyed down to the level of a line joining the posterior margins of the eyelids, and the experiments consisted in observing how long these decerebrate frogs, suspended by the lower jaw, took to withdraw their pelvic extremities from a solution of 1 in 500 sulphuric acid in tap water - before and after the immersion of the right foot in a solution of the extract of Homeria collina. In every case the limbs were immersed in the acid solution up to the same point, (the level of the ankle joint) and the left foot was placed in the acid after the right had been withdrawn. Immediately on withdrawal the foot was washed in tap water.

The following experiment indicates the results obtained:-

Experiment XVII. Homeria collina extract (1 in 100).

The right foot of a large Rana esculenta was immersed in a solution of the extract in distilled water, 1 part in 100, to a point midway between the ankle and the knee joints for such periods as are noted below; during the same periods the left foot was placed in distilled water to the same level.

Interval/

Interval.	Time after which the foot was withdrawn from the acid solution.		Notes.
	Right foot	Left foot	
Before immersion in the poison.			
45 minutes	4 seconds	4 seconds.	
5 "	4 "	3 "	
*			The right foot was now immersed in the poison and the left foot in distilled water for 30 minutes, during which time there was no evidence of the poison irritating the skin.
After 1st immersion in the poison.			
2 minutes	4 seconds	3 seconds	
5 "	3 "	3 "	
15 "	3 "	3 "	
30 "	3 "	3 "	
45 "	3 "	3 "	
60 "	3 "	3 "	
75 "	3 "	3 "	
125 "	3 "	3 "	
*			Each foot was again immersed as above, but this time for one hour.
After 2nd immersion in the poison.			
5 minutes	3 "	4 "	
15 "	3 "	3 "	
20 "	4 "	4 "	
30 "	3 "	3 "	
60 "	4 "	4 "	

Immersion for 1 hour in a freshly prepared 1% sloution of the extract, therefore, does not affect the/

the sensory nerve-ends in the skin of the frog's leg. Similar results were obtained with more dilute solutions (e.g. 1 in 500).

In order to test further the action on sensory nerves, solutions of the extract in distilled water were applied to the surface of the rabbit's eyeball. Even when 0.1 c.c. of a freshly prepared 1% solution was placed on the right eyeball neither increase nor diminution of the sensibility of the right cornea (as compared with the left) was manifested during six hours succeeding the instillation, nor did the transverse diameters of the two pupils differ during that time.

From these experiments it appears that the alcoholic extract of Homeria collina does not affect the peripheral terminations of afferent nerves, and that its local application to the eyeball does not affect the size of the pupil.

c. Motor Nerves.

Among the general effects following the administration of the extract of Homeria collina there is none which points to an important action upon the peripheral terminations of motor nerves. In experiment XVI, 5 hours after the subcutaneous administration to a frog of three times the minimum-lethal/

lethal dose (and $3\frac{1}{2}$ hours after the heart was arrested), equal contractions of their gastrocnemii were obtained (with the secondary coil at 370 m.m.) on direct stimulation of that sciatic nerve which had been exposed to the influence of the poison and of the sciatic nerve of the opposite side which had been protected from contact with the extract.

To investigate further the action on motor nerves a series of experiments was performed with nerve-muscle preparations, consisting of the frog's gastrocnemius with a long piece of the sciatic nerve attached, isolated and placed in Ringer's solution. The nerve trunk of one preparation was placed in a suitable vessel with the muscle of a second preparation, while the muscle of the first preparation and the nerve of the second occupied a contiguous vessel. In one of these vessels the Ringer's solution was replaced by a solution of the extract in Ringer. By means of a 4-volt accumulator and a Du Bois Reymond's induction apparatus the minimal single break shock required to cause contraction of each muscle (a) through its nerve and (b) on direct stimulation of the muscle, was noted repeatedly before and after immersion in the solution of extract of Homeria collina, as in the following experiment.

Experiment XVIII/

Experiment XVIII. Immediately after pithing a frog (Rana temporaria) weighing 55 grams, two nerve-muscle preparations were made. In the following table these preparations are designated A and B. The nerve of A and the muscle of B were each immersed in 3 c.c. of a 1 in 500 solution of the extract in Ringer's solution. Before this immersion the normals were taken.

Interval.	Minimal Single Break Shock required.				Notes.
Before poisoning	Muscle A.	Muscle B.	Nerve B.	Nerve A.	
5 minutes	190 m.m.	180 m.m.	500 m.m.	450 m.m.	
*	Control	Poisoned	Control	Poisoned.	Muscle B and nerve A were immersed in a solution of the extract in Ringer. (1 in 500)
After Poisoning.					
5 minutes	190	180	490	450	
10 "	200	190	490	450	
15 "	200	190	480	440	
20 "	190	190	490	450	
30 "	200	180	480	430	
40 "	200	180	470	430	
60 "	200	180	470	440	
70 "	200	180	460	440	
80 "	200	180	420	450	
90 "	210	190	430	450	
100 "	200	170	410	470	
120 "	210	170	420	460	
140 "	210	170	420	470	
150 "	210	170	420	450	
160 "	200	170	410	440	
175 "	210	170	400	400	
190 "	210	170	400	400	
210 "	200	180	400	400	
220 "	210	180	410	400	
230 "	210	100	90	390	
240 "	200	70	0	380	
250 "	200	60	0	380	
260 "	200	70	0	380	
270 "	200	70	0	380	
300 "	200	40	0	360	
18 hours	190	0	0	0	
20 "	160	0	0	0	

During the experiment no visible fibrillary twitches occurred either spontaneously or after stimulation. At the termination of the experiment both muscles were acid to litmus paper.

This experiment shows that, when a muscle and its nerve ends are poisoned with a strong solution of extract (1 in 500), the response of the muscle to direct electrical stimulation is not markedly affected within 3½ hours of immersion but that it becomes rapidly diminished after that time and is completely abolished several hours before the unpoisoned muscle ceases to respond to direct stimulation. The experiment also indicates that the response of the poisoned muscle to electrical stimulation of its nerve trunk is unaffected during 3½ hours after poisoning and that very soon thereafter powerful electrical stimulation of its nerve trunk is ineffective; one hour later weak electrical stimuli applied to the nerve trunk of the unpoisoned muscle cause it to contract. The poisoned muscle responds to direct electrical stimulation of its fibres for at least sixty minutes after stimulation of its nerve trunk produces no effect.

When the extract is used in a dilution of 1 in 1,000 none of the above differences between the poisoned and the unpoisoned muscles occurs within 10 hours.

By the use of a 1% solution of the extract (a distinctly/

distinctly acid solution) the following points were observable:-

During the first ten minutes after immersion fibrillary twitches occur in both muscles, but especially in the unpoisoned muscle the cut end of whose nerve trunk is immersed in the poison; only the proximal end of the poisoned muscle is affected; the effects produced by a 1 in 500 solution occur more rapidly with a 1% solution, and, in addition, fibrillary twitches sometimes appear in the poisoned muscle after stimulation.

D. Effects on Skeletal Muscles.

In experiments to determine the general effects of the extract of Homeria collina on frogs it was noted that marked rigidity of the skeletal muscles occurred which was succeeded by flaccidity, and that the muscles were contractile for a short time after death though slightly acid in reaction.

In rabbits and cats fibrillary twitches of the skeletal muscles appeared (though not constantly) and there was distinct muscular weakness; soon after death the muscles were still contractile and faintly acid in reaction.

In Experiment XVI it was observed that muscles unprotected from the action of the extract were distinctly/

distinctly acid at a time when muscles protected from it were doubtfully acid in reaction, and Experiment XVIII shows that a skeletal muscle (isolated and placed in a solution of the extract) becomes non-contractile several hours before an isolated muscle placed in Ringer's solution, and, further, that immersion in a 1 in 500 solution of the extract does not cause visible fibrillary twitches in skeletal muscle.

In order to obtain graphic records of some of the changes produced in muscle, a series of experiments was performed in which two muscle preparations (of the gastrocnemii of Rana temporaria) were used. The tendo Achilles and that portion of the femur from which the muscle springs were retained. Each preparation was placed in a small glass cylinder, closed at its lower end by a cork. Through this cork passed a piece of stout platinum wire whose upper end was hooked so as to fix the attached portion of the femur; the other end was connected with one pole of an induction coil. The tendo Achilles of the muscle was attached to a light lever writing on a smoked surface. To complete the electrical circuit a piece of fine platinum wire was hooked into the tendo Achilles and its free end was connected with the other pole of the induction apparatus. Single break shocks from a Du Bois Reymond's apparatus and a 4-volt accumulator were used as stimuli, and the wires from the two muscles/

muscles were so connected that each stimulus passed through both muscles and in the same direction along each muscle. The muscles were surrounded by Ringer's solution. One was used as a control throughout the experiment and the solution surrounding the other was replaced by a solution of the extract in Ringer. The position of the secondary coil is indicated on the tracings.

Out of a number of experiments with different amounts of the extract the following are selected as typical of the action of Homeria collina.

Experiment XIX. Effect on the skeletal muscle of Rana temporaria, of extract of Homeria collina newly dissolved in Ringer's solution (1 in 1,000) Plate II.

Muscle A is the control and muscle B the poisoned muscle. Normal curves were taken 10 minutes (Fig.1) and 2 minutes (Fig.2) before poisoning B.

Interval after poisoning.	Notes.	No. of Figure.
6 minutes	Stimulation at 80 m.m. - no obvious change.	3
11 "	Stimulation at 80 m.m. ditto.	4
46 "	" " " " ditto.	5
83 "	" " " " ditto	6
2 hrs.23 min.	" " " " ditto.	7
2 hrs/		

Interval after poisoning.	Notes.	No. of figure.
2 hrs.43 min.	The lever of B had risen a little, owing to shortening of the muscle. No fibrillary twitches were visible and no jerking of the lever could be seen. The lever continued to rise very gradually but there was no visible movement of the muscle up to 3 hours 28 minutes after immersion in the poison, at which time another stimulus was passed through both muscles.	
3 hrs.23 min.	The tracing shows (at S) the height to which the lever of B had risen as a result of gradual shortening of the muscle. (11 m.m.)	8.
3 hrs.28 min.	After stimulation at 50 m.m. and contraction, the lever of B fell a little lower. 5 minutes later the lever had risen to its former level, but no fibrillary twitches had appeared in the muscle during that shortening.	9
3 hrs.36 min.	The same phenomena followed stimulation at 40 m.m.. The lever of B fell lower than at 3 hrs.28 min., it did not rise to its former level again nor were fibrillary twitches visible in muscle B.	10
4 hrs.40 min.	After stimulation at 40 m.m. muscle B gave a very slight contraction while the control A contracted well.	11
5 hrs.30 min.	After stimulation at 20 m.m. the contraction of muscle B was feeble, and the contraction of muscle A was still fairly good.	12
16 hours	Stimulation at zero m.m. produces only a feeble contraction of B or of A, but A contracts better than B.	13.

16 hours after immersion of B in the poison, both muscles were distinctly acid to litmus paper and muscle B was paler than muscle A.

Experiment XX. Effect on the skeletal muscle of Rana temporaria, of extract of Homeria collina dissolved in Ringer's solution (1 in 1,000). This dilution was made from a 1% solution which had been kept in contact with air in a stoppered bottle at laboratory temperature (about 55°F) for two months. Plate III.

Muscle A is the control and muscle B the poisoned muscle. Normal curves were taken 12 minutes (Fig. 1), and 1 minute (Fig.2) before poisoning B.

Interval after poisoning.	Notes.	No. of Figure.
5 minutes	Stimulation at 100 m.m. no obvious change.	3
10 "	Stimulation at 80 m.m. ditto.	4
30 "	" " 80 m.m. ditto.	5
1 hour	" " 80 m.m. the contraction of B was larger	6
1 hr.28 min.	During the last 15 minutes the lever of B had gradually risen to 7½ m.m. above its original level (S), but no fibrillary twitches were visible in the muscle.	7
1 hr.33 min.	After stimulation at 80 m.m. and contraction, the lever of B fell to a lower level, and then very slowly rose to a still higher level, and fibrillary twitches were visible after stimulation.	8.
1 hr.40 min.	As in last note	9
2 hours/		

Interval after Poisoning.	Notes.	No. of Figure.
2 hours.	After contraction the lever of B did not fall to a lower level than before stimulation.	10.
6 hours.	Stimulation at 40 m.m., muscle B gave no response while muscle A responded well.	11.
6 hrs.10 min.	Stimulation at zero m.m., muscle B gave a feeble response, muscle A a good one.	12.

From these and other experiments it appears that immersion of a skeletal muscle in a solution of extract of Homeria collina is followed after a considerable time by shortening of the muscle and consequent elevation of the lever (see Plate II. fig. 8 13 and Plate III. fig 7 to 12). This shortening is not accompanied by visible fibrillary twitches. If the muscle be now stimulated to contract, it becomes more elongated on relaxing, but the lever does not reach the original abscissa (see Plate II - fig. 9 and 10. Plate III - fig. 8 and 9). This extra elongation is gradually overcome by shortening of the muscle during which coarse fibrillary twitches are visible if old solutions of extract be used. The next change observed is that the muscle has relaxed a little (Plate II - fig. 11 to 13. Plate III fig. 12) though still much shortened, and that its power/

power of contraction is now feeble. A small increase in the height of contraction may occur before the stage of shortening comes on (Plate III - fig. 6) but this effect is inconspicuous and inconstant, as are changes in the form of the muscle curve. The poisoned muscle fails to respond to strong electrical stimuli long before the unpoisoned muscle does so. (Plate II - fig. 11, and Plate III - fig. 11.)

An interesting fact brought out in the two experiments described is that old solutions of the extract, while producing the same effects on muscle as solutions newly prepared, cause these effects to occur more rapidly and also produce visible fibrillary twitches in the poisoned muscle.

E. Effects on the Circulation.

a. Heart.

In experiments to determine the general action of Homeria collina the most striking effects (both in cold-blooded and in warm-blooded animals) were those on skeletal and cardiac muscle. The heart muscle seemed to be particularly sensitive to the influence of the extract, and observation of the cardiac impacts revealed that these were at first increased in rate, in force and in extent; later they became irregular in rate and in force, and just before death they were feeble, irregular and difficult to determine. When the thorax was opened immediately after death, it was usually found that the heart's ventricle was motionless, pale and firmly contracted; it did not respond to direct mechanical stimulation and soon became distinctly acid in reaction. In the case of warm-blooded animals the left ventricle showed these characters more distinctly than did the right. Experiments to determine more exactly the extent and the nature of this action upon the heart now fall to be considered.

In the first series of experiments the extract dissolved in Ringer's solution was applied to the outer/

outer surface of the heart of Rana temporaria, definite quantities of standard solutions being instilled within the pericardial sac. The procedure was as follows. The brain was destroyed anterior to a line joining the posterior margins of the eyelids. The frog was then pinned down on its back and the heart was fully exposed without damaging the pericardial sac. When the sac was damaged, the animal was discarded. A small portion of the anterior wall of the sac over the auriculo-ventricular junction was removed with scissors. By means of a hypodermic syringe from which 100 drops of Ringer measured 1 c.c., 5 or 10 drops of the standard solutions used (= 0.05 c.c. and 0.1 c.c.) were instilled within the pericardial sac through the artificial opening. During this process the cut edge of the sac was seized with fine forceps and the upper part of the sac gently raised from the surface of the heart. After the instillation, the pericardium was not released until several contractions of the heart had distributed the solution all over its surface. These quantities of fluid were easily retained within the pericardial sac of frogs weighing 20 grams and upwards, and the heart's movements did not cause the solution to overflow. In order to keep the tissues moist the frog was laid on wet filter-paper and was covered by a glass funnel.

In such experiments upon frogs without the application/

application of any venom it was found that the heart's movements preserved their chief characteristics for 48 hours after the heart was exposed - the contractions continued to be regular in time; ventricular systole continued to occupy two-thirds of the ventricular cycle, and the ventricle was of a pale pink colour in systole and never white; the rate of contraction fell from 8 to 6 per seconds and the ventricle did not become quite so small in systole as at the beginning of the experiment; general reflexes could still be elicited.

Experiment XXI. 0.005 gram of extract instilled within the pericardial sac of Rana temporaria.

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
Before poisoning 1 minute	7	7	Contractions regular and powerful; ventricular systole occupies two thirds of the ventricular cycle; in diastole the ventricle becomes uniformly dark red in colour and in systole it is pink and moderately small. Auricular systole and ventricular diastole are synchronous and the auricular movements are good.
*			0.005 gram of extract in Ringer's solution was inserted inside the pericardial sac. All the heart's chambers were at once arrested in full diastole for 3 seconds.
After poisoning 1 minute/			

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
1 minute	8	8	Contractions regular and powerful and as before poisoning except that perhaps the ventricle becomes a little smaller and paler in systole.
10 "	8	8	As at 1 minute after poisoning.
27 "	5	5	Contractions regular and powerful; ventricular systole and diastole each occupy half of the ventricular cycle and the ventricle begins to dilate before auricular systole occurs. The auricular movements are good. Ventricular systole is more complete and deliberate, and there is a tendency for the ventricle to pause momentarily in systole.
37 "			The heart was arrested for 5 seconds with the auricles somewhat dilated and the ventricle moderately contracted. Thereafter the auricles began to beat but only irregular patches of the ventricle dilated and these at once contracted again.
40 "	4	4	Regular but not powerful. The auricular movements are feeble; the ventricle is small and pale; it expands momentarily at each auricular contraction but does not become darker in colour.
42 "			For 60 seconds the heart was arrested with the auricles dark and dilated and the ventricle small, pale and well/

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
<u>After poisoning</u>			well contracted. Then the auricles gave a single powerful contraction, and propelled their contents into the ventricle which dilated well and rapidly contracted again. In the next 60 seconds 28 auricular and 28 ventricular contractions occurred, but the extent of the ventricular diastole which accompanied each contraction of the auricles became progressively smaller and the next seven auricular contractions failed to propel any blood into the ventricle; another pause for 50 seconds followed and another similar series of contractions.
47 minutes			Notes as at 42 minutes.
54 "			The heart was arrested with the auricles very large and dark, and the ventricle small and pale. At irregular intervals the auricles contracted with considerable power but they never succeeded in propelling any part of their contents into the ventricle. The ventricle relaxed a little at each auricular systole but without changing colour, and it at once contracted again. From time to time feeble general struggles occurred, but these did not change the condition of the heart.
62 "			The heart is motionless; the auricles are large and dark; the ventricle is small and pale. From time to time general struggles occur, but no movement of the heart results from these.

Interval.	Contractions per 10 seconds. Auricles.	Ventricle	Notes.
After poisoning.			
74 minutes			The heart is still motionless; the veins, sinus and auricles are very dark and dilated; the ventricle is small and pale. The auricles respond feebly to mechanical stimulation; the ventricle is inexcitable.
75 "			A section of the ventricle is neutral or indistinctly acid to litmus paper. General reflexes cannot be elicited.

In this experiment the application of the extract to the surface of the heart was immediately followed by temporary arrest of that organ in diastole. During the next ten minutes there was an inconspicuous increase in the rate of contraction without alteration in the rhythm and the ventricular contractions were more complete. This stage was followed by distinct slowing of the rate of contraction, accompanied by and due (at least in part) to an alteration in the ventricular cycle, viz., ventricular relaxation began before the auricles contracted and the diastolic pause was prolonged. There was as yet no irregularity in the rate of contraction; the movements of the auricles were good and the contractions of the ventricle much more powerful and deliberate. After this stage great irregularity in the rate of contraction occurred owing/

owing to prolonged pauses at irregular intervals during which the auricles were dilated and the ventricle contracted. Finally the contracted ventricle resisted all the efforts of the auricles to dilate it and it became inexcitable before the auricles did so. At this time it was very pale and small and a section of its muscle was doubtfully acid to litmus paper.

Experiment XXII. 0.0025 gram of extract instilled within the pericardial sac of Rana temporaria.

Interval.	Contraction per 10 seconds. Auricles.	Ventricle.	Notes.
Before poisoning			
30 minutes	6	6	Contractions regular and powerful; ventricular systole occupies two thirds of the ventricular cycle; in diastole the ventricle becomes a uniform dark red and in systole it is pink and moderately small. Auricular systole and ventricular diastole are synchronous; the auricles expand and contract well, but do not empty themselves completely in systole.
3 "	5	5	Ditto.
*			0.0025 gram of extract in Ringer's solution was inserted inside the pericardial sac. All the heart's chambers were at once arrested in full diastole for about 3 seconds.

After/

Interval.	Contraction per 10 seconds. Auricles.	Ventricle.	Notes.
After poisoning.			
2 minutes	5	5	As last described.
3 "			The heart paused with all chambers in diastole for a second; then the auricles began to beat feebly and the ventricle well. Gradually the auricular beats became normal again.
19 "			The heart paused with all chambers in diastole for seven seconds and then spontaneously resumed beating.
21 "			7 auricular and 7 ventricular contractions in 20 seconds, regular and powerful. Ventricular diastole now occupies half of the ventricular cycle and precedes auricular systole. The auricles are better filled and propel their contents into the ventricle more completely; the ventricle relaxes rapidly but remains in full diastole longer; it is larger in diastole and smaller and paler in systole. Ventricular systole is more deliberate.
23 "			The heart paused with all chambers in diastole for three seconds and then resumed as at 21 minutes.
25 "			Pause as above for 5 seconds, and spontaneous recovery.
29 "			As last noted.
30 "	4	4	Regular and powerful contractions with characters exactly as at 21 minutes.

35/

Interval.	Contraction per 10 seconds. Auricles.	Ventricle.	Notes.
After poisoning 35 minutes			7 auricular and 7 ventricular contractions in 20 seconds; characters as at 21 minutes.
37 "			All the heart's chambers were arrested in diastole for 9 seconds, except for a single auricular and ventricular contraction. Then 12 [illegible] regular spontaneous contractions of all chambers occurred, followed by another pause in diastole for four seconds.
41 "	3	3	Contractions regular and powerful; the auricles tend to remain in diastole for longer periods and the ventricle follows them.
49 "	3	3	Ditto.
64			4 auricular and 4 ventricular contractions in 20 seconds; regular and powerful. Ventricular diastole continues to occupy half of the ventricular cycle; the auricles fill and empty well; the ventricle dilates well and contracts very well, tending to pause in extreme systole.
79 "			As last noted
90 "			All the heart's chambers paused in full diastole for 20 seconds. Then they gave a single contraction and again paused in diastole for 10 seconds and thereafter they resumed contractions as at 64 minutes.

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
After poisoning.			
99 minutes	4	4	Characters as at 21 minutes.
109 "			All chambers arrested in full diastole for 60 seconds. Then the auricles and ventricle gave a single complete contraction and again paused in full diastole for 15 seconds. Then the auricles and ventricle gave a single contraction and paused for 10 seconds; then a single contraction and paused for 5 seconds and thereafter the heart beat regularly and powerfully at 3 per 10 seconds.
119 "			4 auricular and 4 ventricular contractions in 60 seconds at irregular intervals, owing to diastolic pauses of varying length. Each ventricular contraction is very complete and rather abrupt; the dilatation of the ventricle is more gradual than it was.
124 "			7 auricular and 7 ventricular contractions in 60 seconds. Notes as last recorded.
139 "			Ditto.
149 "			8 auricular and 8 ventricular contractions in 60 seconds at almost regular intervals. After systole the ventricle does not become uniformly dark for 4 to 6 seconds. i.e. gradual relaxation.
164 "			As last noted.

174/

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
<u>After poisoning</u> 174 minutes			Ditto. The relaxation of the ventricle is distinctly more gradual; its contraction is rapid and complete with a tendency to pause in systole. The auricular movements are good; auricular relaxation is rapid and there is a regular prolongation of the auricular diastolic pause. During the next 18 hours observations were made from time to time; the heart's movements preserved the above characters almost unchanged, but the regular auricular diastolic pause became longer and distinct pauses gradually appeared at the end of ventricular systole. These changes caused a further slowing of the heart's rate.
24 hours			2 to 4 contractions of all chambers in 60 seconds owing to irregular prolonged diastolic pauses. The auricles dilate well but do not contract powerfully or completely. The ventricle contracts well and rapidly; it relaxes very slowly.
28 "			The heart was finally arrested. The auricles were dark, dilated and inexcitable. The ventricle was moderately dark and dilated and was contractile. After numerous mechanical stimuli it became pale, small and firmly contracted. The reaction of a section of the ventricular muscle to litmus paper was doubtful.

In this experiment a smaller quantity of the

extract/

extract was applied to the surface of the heart. Immediately after the application, all the heart's chambers became temporarily arrested in full diastole. Thereafter the rate of the heart became slowed owing to the more complete and deliberate systolic movements of the ventricle and to the prolongation of the ventricular diastolic pause. The occurrence of complete arrest of the heart's chambers in diastole at irregular intervals and for periods of several seconds duration made the heart's rate at times irregular. The auricular movements were more complete as well as the ventricular. At a later period (41 minutes after poisoning) there was a regularly occurring prolongation of the auricular diastole as well as of the ventricular, while long pauses in diastole continued to affect all the chambers from time to time. In 174 minutes after poisoning, these irregular diastolic pauses had disappeared; the heart's contractions were much slower than before poisoning, and were regular in time; the auricles contracted well and rapidly and there was a regular prolongation of the auricular diastolic pause; the ventricle contracted very powerfully and rapidly; it paused momentarily in systole and its relaxation was distinctly more gradual. These important characters were preserved for many hours. Finally all the heart's chambers were arrested in/

in diastole; the auricles were inexcitable, while the ventricle continued to respond to direct mechanical stimulation and went into complete systole after repeated stimulation. The reaction to litmus paper of the ventricular muscle at this time was doubtful.

Influence of the Vagus.

With a laboratory temperature of 18° to 20° C. it was found that weak electrical stimulation of the exposed vagus nerve in the frog caused complete arrest of the heart in extreme diastole for several seconds. It was established by numerous experiments that, within three minutes of the application of a solution of Sulphate of Atropine (in Ringer's solution) to the surface of heart of Rana temporaria, powerful electrical stimulation of the vagus produced no effect. During two hours after 0.000025 gram of Atropine Sulphate was instilled within the pericardial sac the strongest stimulation (from a Du Bois Reymond's apparatus and a single Bi-chromate cell) of the isolated vagus nerve produced no effect on the heart, except slight acceleration in some instances. After this interval electrical stimulation of the vagus arrested the heart in diastole, but a second application of the same dose of Atropine Sulphate abolished the vagus-action for other two hours.

The/

The following experiment is one of a series in which Atropine Sulphate was applied to the surface of the frog's heart before the application of a solution of the extract of Homeria collina. The object of these experiments was to ascertain whether paralysis of the Vagus by Atropine prevented or modified the effects produced by the extract.

Experiment XXIII. 0.0025 gram of extract instilled within the pericardial sac of Rana temporaria after the instillation of 0.000025 gram of Sulphate of Atropine.

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
Before poisoning			
20 minutes	8	8	Contractions regular and powerful; ventricular systole occupies two thirds of the ventricular cycle; in diastole the ventricle becomes of a uniform dark red colour and in systole it is small and moderately pale. The auricular movements are good.
7 "	8	8	Ditto.
*5 "			0.000025 gram of Atropine Sulphate in Ringer's solution was inserted within the pericardial sac.
2 "	7	7	Notes as at 20 minutes before poisoning, except that the auricles do not empty quite so well in systole.
*			0.0025 gram of extract in Ringer's solution was inserted/

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
			inserted within the pericardial sac. The heart did not become arrested in diastole.
After poisoning.			
3 minutes	8	8	Contractions regular and powerful; rhythm unchanged; the auricles expand more in diastole but do not contract more completely; the ventricle is larger in diastole and moderately small and pale in systole.
7 "	8	8	Regular and powerful contractions; ventricular diastole now occupies half of the ventricular cycle; the auricles dilate well and contract rapidly; the ventricle dilates rapidly and before auricular systole; its diastolic pause is prolonged; ventricular systole is more deliberate than before and the ventricle becomes smaller and paler than before.
13 "	6	6	Contractions regular; ventricular systole again occupies two thirds of the ventricular cycle; the auricles expand well and contract more completely; the ventricle dilates well and contracts deliberately, becoming very small and pale in systole.
20 "	6	6	Notes as above.
25 "	5	5	Ditto.
35 "	4	4	Characters as last described except that ventricular diastole occupies half of the ventricular cycle and precedes/

Interval.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
<u>After poisoning.</u>			precedes auricular systole.
45 minutes	4	4	Ditto.
55 "			All the heart's chambers are from time to time arrested in full diastole sometimes for 30 seconds. After such pauses the auricles contract first.
60 "			The regularity of the rate is interrupted by diastolic pauses of irregular length as at 55 minutes. Otherwise the contractions are as at 35 minutes.
76 "			Ditto.
103 "			Pauses of about 15 seconds with all chambers in full diastole alternate with pairs of very complete and rapid contractions of all chambers.
*108 "			Again applied 0.000025 gram of Atropine Sulphate to the surface of the heart.
111 "			Series of 2 to 5 very complete cardiac contractions alternate with pauses in diastole lasting from 5 to 15 seconds. The auricles always resume contracting before the ventricle.
115 "			Inserted 0.00005 gram of Atropine Sulphate within the pericardial sac.
117 "			Notes as at 111 minutes.
120 "			Series of 1 to 6 powerful cardiac contractions alternate with long pauses during which all the chambers are/

Intercal.	Contractions per 10 seconds. Auricles.	Ventricle.	Notes.
<u>After poisoning.</u>			are in full diastole.
128 minutes			Ditto.
143 "			Ditto.
170 "			11 auricular and 11 ventricular contractions in 60 seconds at irregular intervals owing to long diastolic pauses. The auricular movements are good. The ventricle rapidly becomes very small and pale but there is no tendency for it to pause in systole. It dilates rapidly and then pauses in full diastole.
230 "			Exactly as at 170 minutes.
5 hours			Ditto.
6 hrs.10 min.			10 auricular and 10 ventricular contractions in 60 seconds and at more regular intervals owing to greater regularity in the length of the diastolic pauses. The chambers fill and empty well and the ventricle is very small and pale in systole.

In this experiment the amount of extract applied to the surface of the heart was the same as in Exp. XXII, but it was applied after Sulphate of Atropine. In the present experiment the heart was not arrested in diastole immediately after the application of the extract, but otherwise the results of instilling Homeria collina into the pericardial sac were similar to/

to those in Exp. XXII., viz., the rate was slowed; the movements of the auricles and ventricle, especially ventricular systole, were increased in amplitude and power, and long pauses in diastole occurred at irregular intervals.

After these changes had appeared a second application of the same amount of Atropine Sulphate and later the application of twice that amount produced no alteration in their characters.

These three experiments (XXI, XXII, XXIII) demonstrate the following important effects of Homeria collina on the frog's heart:— The rate of the contractions is slowed; the movements of the auricles and ventricle are increased in amplitude and the systolic movements are increased in power, ventricular systole especially becoming more complete and more deliberate. The slowing of the heart's rate is partly due to this increased range and deliberation of movement, but lengthening of the diastolic pause is another factor to account for it. With the smaller doses the heart becomes arrested with all its chambers in diastole, though the ventricle will still pass into complete systole in response to repeated mechanical stimulation; larger doses arrest the ventricle in systole and the auricles are then distended with blood. After the final arrest of the heart the ventricular muscle/

muscle tends to become acid in reaction.

The prior application of Sulphate of Atropine prevents the occurrence of that diastolic arrest of the heart which follows immediately upon the application of the extract, but it does not prevent, nor indeed modify, the other effects of Homeria collina; nor does the application of Atropine after the extract abolish or modify those effects which have already appeared.

Heart Perfusion through the Hepatic Vein.

In order to obtain graphic records of the movements of the frog's heart a series of experiments was performed in which the extract dissolved in Ringer's solution was perfused through the heart by means of a canula tied into the hepatic vein, the aortae being cut to allow the fluid to escape readily. The canula was connected with two reservoirs, the one containing only Ringer's solution and the other a definite quantity of the extract in Ringer's solution. The record was obtained by means of a light lever, one end writing on a revolving smoked surface and the other end being attached superficially to the apex of the ventricle by a small hook.

By means of Marriotte's flasks the fluid in the reservoirs was kept at a constant level of $1\frac{1}{4}$ inches above the outlet of the canula. Rana temporaria was used and the brain was destroyed. Before the solution of extract was perfused, the heart movements with Ringer's solution were recorded.

Experiment XXIV. Heart perfusion through the hepatic vein with extract of Homeria collina in Ringer, (1 in 500) after Ringer's solution alone. Plate IV.

Interval/

Interval	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
1 minute before extract.	6 m.m.	29	1	The extent of the movements is due almost entirely to the ventricle.
*				Perfusion of extract in Ringer commenced (1 in 500).
3 min. after *	5 "	28	2	Ditto.
10 "	6 "	25	3	This slowing is due to occasional slight delay in the occurrence of auricular systole after ventricular diastole. At such times the ventricular relaxation is greater and the auricles are better filled.
18 "	8 "	14	4	The delay in the occurrence of auricular systole is now almost regular. The ventricle and the auricles relax more in diastole and the auricular systolic movement now forms a distinct part of the upstroke. The ventricular systolic movement is also increased.
23 "	10 "	1 to 8	5	Long diastolic pauses occur. The auricular systolic movement is $2\frac{1}{2}$ m.m. and the ventricular $7\frac{1}{2}$ m.m.
35 "	11 "		6	The diastolic pauses last for about 2 minutes. Soon after this the heart became arrested in diastole and then the ventricle slowly passed into final systole.

After the perfusion of the extract in this experiment the rate of the heart is slowed. For some minutes the extent of the ventricular contraction is diminished, but thereafter the extent of the systolic and diastolic movements of the auricles and ventricle is considerably increased; the diastolic pause is greatly lengthened. Within an hour the heart is paralysed in diastole and the ventricle then slowly passes into systole.

Experiment XXV. Heart Perfusion through the hepatic vein with extract of Homeria collina in Ringer (1 in 5,000) after Ringer's solution alone. Plate V.

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
1 minute before extract.	9 m.m.	30	1.	The record of auricular systole occupies 2 m.m. at the bottom of the upstroke.
*				Perfusion of extract in Ringer commenced (1 in 5,000).
1 minute after *	7 "	30	2	The diminished amplitude is due to the ventricular contraction being less complete.
6 "	9 "	29	3	Ventricular systole back to normal.
15 "	11 "	26	4	The record of auricular systole occupies the first 3 m.m. of the upstroke; the other 8 m.m. is ventricular.
30 "/				

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
30 minutes after *	8 m.m.	21	5	The auricular systole occupies 5 m.m. of the upstroke, and the ventricular 3 m.m. This is due to the ventricle remaining in semi-systole instead of expanding. The diastolic pause is longer.
40 "	6 "	15	6	The ventricle relaxes only a little in diastole and the auricles are beginning to fail. The diastolic pause is well marked.
50 "	5 "	12	7	Ditto.
63 "			8	The auricles cease to beat and the ventricle slowly passes into complete systole.

This experiment also shows a diminution in the extent of the ventricular contraction immediately after the perfusion of the extract begins. Thereafter the rate of contraction is slowed, the diastolic pause is lengthened and the movements of the auricles and ventricle were increased in extent; but the ventricle passes into permanent systole within 65 minutes.

Experiment XXVI. Heart Perfusion through the hepatic vein with extract of Homeria collina in Ringer (1 in 10,000) after Ringer's solution alone. Plate VI.

Interval/

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
2 minutes before extract.	11 m.m.	20	1.	The auricular contraction occupies the first 2 m.m. of the upstroke. There is a short diastolic pause.
*				Perfusion of extract in Ringer commenced (1 in 10,000)
1 minute after *	10 "	20	2	Ventricular systole is somewhat less complete.
10 "	10 "	20	3	Ditto. Auricular systole occupies 2 m.m.
30 "	11 "	25	4	Auricular systole occupies 3 m.m. and the diastolic pause has disappeared. The short relaxations seen in the figure are due to the auricles contracting while the ventricle is only partly relaxed and thus causing another contraction of the ventricle.
40 "	11 "	25	5	Auricular systole occupies 3 m.m. and the diastolic pause has disappeared.
1 hour	12 "	25	6	Ditto. The ventricular systolic movement is also larger. Every fourth relaxation is cut short by a rapid contraction of the auricles followed by the ventricle.
1hr.15 min.	12 "	19	7	Auricular systole occupies 3 m.m. The first part/

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
				part of the figure shows the same condition as fig.6, but thereafter every fourth beat is missed instead of being accelerated. Still later the figure shows the fourth beat accelerated and missed alternately.
1hr.40 min. after *	12 m.m.	21	8	The first part of the figure shows the fifth beat accelerated and missed alternately; the next part shows it regularly missed, and the last part shows three consecutive accelerations of it.
2hrs.25 min. "	11 "	22	9	Auricular systole occupies 3 m.m. Occasionally a beat is missed.
3 hrs. "	11 "	23	10	Rate of contraction is regular.
4 " "	10 "	22	11	Ditto. auricular systole occupies 3 m.m.
4hr. 30 min. "	9 "	21	12	Rate of contractions regular. The ventricle is going into a condition of diastole.
6hrs. "	8 "	19	13	Ditto.
7 " "	7 "	20	14	Ditto.
8 " "	2 "	21	15	Only the auricles are contracting. The ventricle is arrested in diastole.
8hrs. 6 min."			16	The ventricle has begun to contract again. The first part of the figure show a ventricular contraction following each/

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
				each auricular contraction. Then several auricular contractions precede each ventricular, and still later a second auricular contraction occurs just when the ventricle, having contracted, begins to relax.
8hrs.21 min. after *	10 m.m.		17	After a period of regular contractions during which the auricles relax before the ventricle contracts, the ventricle ceases to respond to the auricular stimulation. Still later the ventricle gives large contractions after varying numbers of auricular contractions. The ventricle relaxes less rapidly at this time.
8hrs.33 min. "	No. 18			A period of auricular contraction only, followed by a series of almost regular heart beats in which the ventricular contractions are large, with a tendency to pause in systole. The ventricular response to auricular systole is delayed.
8hrs.43 min. "	19			Ventricular contractions of 8 m.m., each preceded by three auricular contractions.
9hrs.15 min. "	20			At first only the auricles are contracting: later the ventricle begins to contract. The ventricular contraction is large and relaxation is somewhat delayed. At times the auricles contract during the ventricular relaxation.
9hrs.40 min. "	21			The auricles contract regularly; the ventricle only at irregular intervals.

10 hours/

Interval.	No. of Fig.	
10 hours after *	22	One to three auricular contractions precede each ventricular.
10hrs.10 min. "	23	One to five auricular contractions precede each ventricular.
10hrs.35 min. "	24	The figure shows auricular contractions occurring with a short pause between each pair of contractions and no ventricular response. The ventricle was now arrested in diastole and no longer gave any spontaneous contractions, though it responded to mechanical stimulation 20 hours after the extract was first perfused.

In this experiment the perfusion of the solution of extract was followed by a slight diminution in the extent of ventricular systole for a short time during which the rate of contraction remained unchanged. Then follows an increase in the extent of auricular systole, and a distinct acceleration of the rate, owing to diminution of the auricular diastolic pause.

Auricular systole may occur before the ventricle has completely relaxed, and this may take place at regular intervals. Somewhat later each regularly occurring acceleration of auricular systole is replaced by a pause or missed beat, and now an increase in the systolic movement of the ventricle is apparent. Thereafter the rhythmical acceleration and pause disappear and the ventricle tends to remain in diastole.

As/

As this tendency becomes more marked either the auricles continue to beat regularly for many minutes while the ventricle is motionless and in diastole, or single ventricular contractions occur after several auricular ones. The contraction of the ventricle is then larger and its relaxation more gradual. Finally the ventricle remains in diastole, the auricles continuing to contract. For several hours after this time mechanical stimulation will result in a single ventricular contraction.

Perfusion of the Isolated Ventricle.

The next series of experiments shows the effects of the extract dissolved in Ringer's solution upon the isolated ventricle of the heart of Rana temporaria. The method of perfusion is that employed in Schäfer's Heart Plethysmograph, but the apparatus was adapted so as to record the movements upon a vertical, instead of a horizontal, drum.

Experiment XXVII. Heart Perfusion - frog's isolated ventricle - with extract of Homeria collina in Ringer (1 in 3,000) after Ringer's solution alone. Plate VII.

Interval.	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
2 minutes before extract *	27 m.m.	17	1	A short diastolic and no systolic pause. Perfusion of extract in Ringer (1 in 3,000) commenced. At once there is a diminution in the extent of the systolic contraction.
2 min. after *	30 "	14	2	The increased movement is entirely systolic.
20min."	37 "	9	3	Ditto. The diminished rate is due to larger systolic movement, and to lengthening of the diastolic and of the systolic pauses.
30 min./				

Interval	Amplitude of Excursus.	Contractions in 60 seconds.	No. of Fig.	Notes.
30 min. after *	35 m.m.	11	4	The diastolic and the systolic pauses are shorter than at 20 minutes.
40min."	34 "	13	5	But the extent of the systolic movement is rapidly decreasing and the systolic pause has disappeared.
1 hr. "	22 "	12	6	The extent of the systolic movement has diminished further.
1hr.15 min. "	21 "	8	7	From time to time the ventricle, after relaxing, slowly shortens into a semi-systolic condition and then, giving a feeble contraction, it relaxes normally and beats regularly. After a few regular contractions and relaxations a slight shortening appears before each contraction and progressively increases. When this shortening has attained some considerable degree, the ventricle relaxes a very little instead of rapidly contracting, and then it shortens still more and very slowly. Thereafter it may resume contracting regularly for a time but finally it remains in this condition of moderate systole.

Following the perfusion of the extract in this experiment there is at first a diminution in the extent of ventricular systole. This is rapidly followed by a considerable increase in the systolic movement, a lengthening of the systolic and diastolic pauses and consequently by slowing of the rate. Still later the systolic pause disappears and the extent of the systolic movement and, in a lesser degree, of the diastolic/

diastolic movement is diminished. Finally the ventricle passes slowly into a condition of moderate systolic contraction and, while this is occurring, the record of the movements (fig.7) shows an interesting parallel to movements of skeletal muscle in the same stage of poisoning which are depicted in Plate II - fig. 8 onwards, and Plate III - fig. 7 onwards - i.e., gradual shortening of the muscle, relaxation after contraction to a lower level than that from which the contraction raised the lever, and again gradual shortening.

The results of these perfusion experiments agree with those already obtained from the other experiments which have been described in the immediately preceding section. The graphic records show very clearly the increased range of movements, auricular and ventricular which follows the administration of the extract, while the earlier figures of Plate VI appear to indicate a special action on the auricles and a comparison between the later figures in Plates II and III and the last figure in Plate VII shows a close resemblance between the action of Homeria collina on cardiac and skeletal muscles.

b. BLOODVESSELS.

The action of extract of Homeria collina on the bloodvessels was investigated in frogs (Rana temporaria) whose brain and spinal cord had been destroyed. A canula was tied into the ductus arteriosus and the sinus venosus was divided. Ringer's solution was perfused from a constant level maintained by Marriotte's flasks. When the blood had been entirely washed out of the vessels, the outflow was collected in graduated glass measures and the amount passing through the vessels each minute was recorded. When a normal flow was established, the contents of one of the other Marriotte's flasks containing a solution of extract in Ringer ~~was~~ were perfused instead of simple Ringer's solution. In this way the effect of Homeria collina upon the frog's bloodvessels was obtained. In some experiments a solution of Merck's pure Digitalin in Ringer was perfused after the solution of extract in order to show the difference in their effects.

The following experiments are representative of the series. The level of the solutions above the outlet of the canula was 6½ inches; and the laboratory temperature varied from 52° to 56°F., but was constant during each experiment.

Experiment XXVIII. Ringer's solution only. Plate VIII

Time/

Time in Minutes.	Flow per min. in C.C.	Time in Minutes.	Flow per min. in C.C.	Time in Minutes.	Flow per min. in C.C.
1	3.9	34	3.9	67	3.9
2	3.9	35	3.8	68	4.0
3	4.0	36	3.9	69	3.9
4	4.0	37	4.0	70	3.8
5	4.0	38	4.0	71	3.9
6	3.9	39	3.9	72	3.9
7	4.0	40	4.0	73	3.8
8	4.2	41	3.8	74	3.8
9	4.1	42	3.8	75	3.9
10	4.2	43	4.0	76	3.9
11	4.0	44	4.0	77	3.9
12	4.1	45	4.0	78	3.9
13	3.9	46	4.0	79	3.7
14	4.1	47	3.9	80	3.8
15	4.1	48	3.8	81	3.9
16	4.2	49	3.8	82	3.6
17	4.0	50	4.0	83	3.9
18	4.1	51	4.0	84	3.9
19	4.0	52	3.8	85	3.8
20	4.2	53	3.8	86	3.9
21	4.1	54	3.9	87	3.6
22	4.1	55	3.9	88	3.8
23	4.0	56	3.9	89	4.0
24	3.9	57	3.8	90	3.8
25	4.0	58	3.8	91	3.8
26	4.0	59	3.9	92	3.7
27	4.0	60	3.9	93	4.0
28	4.0	61	3.9	94	3.6
29	3.9	62	3.9	95	3.6
30	4.0	63	3.9	96	3.6
31	4.0	64	3.8	97	3.8
32	4.0	65	3.9	98	3.6
33	4.0	66	3.8	99	3.8
				100	3.7

The frog weighed 37 grams before and 41 grams after this experiment - a gain of 4 grams, owing to oedema. No fibrillary twitches appeared in the muscles during the experiment

Experiment XXIX. Ringer's solution; then extract of Homeria collina in Ringer (1 in 200); then Digitalin in Ringer (1 in 200,000). Plate VIII.

Time in Minutes.	Flow per Min. in C.C.	Time in Minutes	Flow per Min. in C.C.	Time in Minutes	Flow per Min. in C.C.
1	4.7	18	2.2	35	0.9
2	4.9	19	1.7	36	1.1
3	4.7	20	1.8	*Digitalin perfused.	
4	5.0	21	1.7	37	1.1
5	5.0	22	1.5	38	1.2
* Extract perfused		23	1.5	39	1.4
6	4.9	24	1.5	40	1.4
7	4.3	25	1.4	41	1.2
8	4.2	26	1.3	42	1.3
9	3.9	27	1.3	43	1.2
10	3.5	28	1.3	44	1.0
11	3.5	29	1.2	45	1.1
12	3.3	30	1.3	46	0.9
13	3.2	31	1.2	47	0.8
14	2.7	32	1.1	48	0.8
15	2.5	33	1.1	49	0.6
16	2.3	34	1.2	50	0.6
17	2.2			51	0.6

The frog weighed 30 grams before and 36 grams after this experiment - a gain of 6 grams.

The calibre of the blood-vessels was rapidly diminished to a considerable degree, and yet a solution of Digitalin a thousand times weaker rapidly caused a further contraction of the vessels. Immediately after the solution of extract was first perfused very coarse fibrillary twitches appeared in the muscles of the pelvic extremities. These twitches rapidly became more feeble and they disappeared in 15 minutes after the solution of extract was first perfused.

Experiment XXX. Ringer's solution; then extract of Homeria collina in Ringer (1 in 1,000); then Digitalin in Ringer (1 in 200,000). Plate VIII.

Time in Minutes.	Flow per Min in C.C.	Time in Minutes.	Flow per Min. in C.C.	Time in Minutes.	Flow per Min. in C.C.
1	3.5	24	3.4	48	2.8
2	3.5	25	3.5	49	2.9
3	3.5	26	3.5	50	2.8
4	3.6	27	3.3	51	2.8
5	3.6	28	3.4	52	2.6
6	3.5	29	3.4	53	2.8
7	3.7	30	3.4	54	2.5
8	3.4	31	3.4	55	2.6
9	3.7	32	3.4	56	2.5
10	3.6	33	3.4	57	2.5
* Extract perfused.		34	3.4	58	2.3
11	3.7	35	3.5	59	2.5
12	3.7	36	3.3	60	2.5
13	3.8	37	3.4	61	2.3
14	3.7	38	3.6	* Digitalin perfused.	
15	3.7	39	3.4	62	2.4
16	3.6	40	3.5	63	2.2
17	3.7	41	3.3	64	1.8
18	3.7	42	3.2	65	0.7
19	3.5	43	2.8	66	0.7
20	3.4	44	3.0	67	0.6
21	3.6	45	3.0	68	0.4
22	3.5	46	3.1	69	0.3
23	3.4	47	2.9	70	0.3

The forg weighed 34 grams before, and 45 grams after this experiment - a gain of 11 grams.

This solution of extract caused a gradual slight contraction of the vessels, though the outflow was not reduced by one half after 50 minutes. Thereafter a solution of Digitalin 200 times weaker caused a rapid further contraction of the bloodvessels. No fibrillary twitches appeared in the muscles during the experiment.

Experiment XXXI. Ringer's solution; then extract of Homeria collina in Ringer (1 in 10,000); then Ringer's solution alone. Plate VIII.

Time in Minutes.	Flow per Min. in C.C.	Time in Minutes.	Flow per Min. in C.C.	Time in Minutes.	Flow per Min. in C.C.
1	4.9	33	4.9	65	3.8
2	4.8	34	4.9	66	3.9
3	4.8	35	4.9	67	3.8
4	4.9	36	4.9	68	4.3
5	4.8	37	5.0	69	3.9
6	5.0	38	4.5	70	3.9
7	5.0	39	5.0	71	3.7
8	4.8	40	4.7	72	4.1
9	4.7	41	4.2	73	3.8
10	4.9	42	4.8	74	4.1
* Extract perfused.		43	4.2	75	4.0
11	4.8	44	4.4	76	3.9
12	4.9	45	4.3	77	3.7
13	4.9	46	4.1	* Ringer perfused.	
14	5.0	47	4.1	78	3.3
15	4.9	48	4.1	79	3.6
16	4.8	49	4.4	80	3.5
17	4.8	50	4.5	81	3.5
18	4.9	51	4.3	82	3.6
19	4.8	52	4.4	83	3.6
20	4.8	53	4.2	84	3.5
21	4.8	54	4.4	85	3.6
22	4.9	55	4.2	86	3.7
23	4.9	56	4.3	87	3.5
24	4.8	57	4.1	88	3.5
25	4.9	58	4.1	89	3.4
26	4.8	59	3.9	90	3.0
27	4.6	60	4.1	91	3.2
28	4.7	61	4.1	92	3.4
29	4.6	62	4.1	93	3.4
30	4.6	63	4.3	94	3.1
31	4.5	64	4.0	95	3.4
32	4.9			96	3.3

The frog weighed 59 grams before and 72 grams after this experiment - a gain of 13 grams.

The bloodvessels were practically unaffected by this solution of extract, the diminution in the outflow being about one fifth after 75 minutes. No fibrillary twitches appeared in the muscles during the experiment.

From these experiments it appears that extract of Homeria collina causes the frog's blood-vessels to contract, but that this effect is produced definitely only by solutions of a greater concentration than 1 in 10,000. The amount of contraction caused by a solution of even 1 in 200 is less than that produced by Merck's pure Digitalin in a dilution of 1 in 200,000. Contrasted with Digitalin, extract of Homeria collina has a very feeble action on the bloodvessels.

c. Heart and Blood-vessels. (Blood Pressure).

Kymographic experiments were performed in order to determine the effects of the extract on the blood-pressure and the respiratory movements were recorded by means of a double stethograph. The following experiments are selected from this series. In the first of these no other factor than the administration of the extract was introduced; in the second the vagus was stimulated from time to time before and after the administration of Homeria collina; in the third Atropine was administered before the extract; in the fourth a plethysmogram of the intestines was recorded simultaneously with the record of the blood-pressure.

Experiment XXXII. Weight of rabbit, 1950 grams. 0.078 gram of extract of Homeria collina injected into a jugular vein in four equal parts. Each portion was dissolved in 2 c.c. of Ringer's solution and was equivalent to 0.01 gram per Kilogram; and the total dose was equivalent to 0.04 gram per Kilogram.
Plate IX.

Time/

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
Before injection.						
2 min.	90	40	15	3	1	Pulse waves barely 1 m.m.
½ "	90	39	14	3	-	Ditto.
*					2	0.01 gram per kilogram injected intravenously (first injection).
After injection.						
1/3 min.	106	40	13	3	2	Pulse movements barely 1 m.m.
1 "	110	40	14	3	-	
2½ "	118	39	13	3.5	3	Pulse movements 1 m.m.
5 "	107	38	11	4	-	
6 "						0.01 gram per kilogram injected intravenously (second injection).
7 "	110	37	11	4.5	4	Pulse movements 1 m.m.
11 "	76	39	13	3	-	Pulse movements 0.5 m.m.
12½	-	-	14	3	-	The blood pressure rose suddenly from 77 m.m. to 106 m.m. and in 3 minutes slowly fell to 74 m.m. Pulse rate unchanged; pulse movements 0.5 to 1.5 m.m. No evidence of a struggle.
17 "	98	40	15	3	-	
18						0.01 gram per kilogram injected intravenously (third injection).
20 "/						

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
After * 20 min.	140	40	14	2.5	-	The level of the blood-pressure has risen gradually and varies rapidly within narrow limits.
22½ "	144	46	13	2.5	5	The blood-pressure is beginning to fall slowly Pulse movements distinctly irregular in size.
27 "	98	43	12	2.5	-	
27¼ "						0.01 gram per kilogram injected intravenously. (fourth injection).
27½ "	58	41	12	2.0	-	At this time the blood-pressure fell rapidly and remained low.
31 "	30	39	4	1.5	6	Pulse movements very small. Respiratory movements cease before the pulse movements do.
33 "	0	0	0	0	-	

In 36 minutes after the first injection the thorax was opened. The heart was found to be arrested with the left ventricle small, pale and firmly contracted. Electrical stimulation of a phrenic nerve with the secondary coil at 350 m.m. and direct stimulation of the muscle at 200 m.m. caused a contraction of the diaphragm.

In/

In this experiment after the first and third injections the blood-pressure rose, at first rapidly and then more gradually, to a considerable height, (Fig. 2, 3 and 5). Thereafter it fell very slowly, (as in Fig. 5). The second injection was administered while the blood-pressure was still well above the normal level, and on that occasion only a slight rise occurred, (Fig. 4). After the fourth injection the blood-pressure fell rapidly to a low level, and then more gradually it sank to zero, (Fig. 6).

The pulse rate was almost unaffected until just before death. The pulse movements were slightly increased after the first and second injections.

The rate of the respirations was diminished and the extent of the respiratory movements was increased after the first and second injections.

Before the third injection, the rate and the extent of respiration were again normal. After the fourth injection each became diminished, the respirations being finally arrested before the pulse movements (Fig.6) . Respiratory convulsions did not occur towards the close of this experiment, though they may appear when single fatal doses are given.

Experiment XXXIII. Weight of rabbit, 2000 grams. 0.08 gram of extract of Homeria collina injected into a jugular vein in four equal parts. Each portion was dissolved in 2 c.c. of Ringer's solution and was equivalent to 0.01 gram per kilogram; the total dose was equivalent/

equivalent to 0.04 gram per kilogram. The right vagus was cut and its peripheral end stimulated at intervals before and after the administration of the extract.

Plate X.

Time.	Average blood-pressure in m.m. of Hg..	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
Before injection.						
3 min.					1	Vagus stimulated; secondary at 150 m.m: almost no effect.
2 min.					2	Vagus stimulated; secondary at 130 m.m. heart slowed and blood-pressure fell, temporarily.
1 "	113	37	10	1.5	3	Pulse movements barely 1 m.m.
*						0.01 gram per kilogram injected intravenously (first injection.)
After *						
1 min.	132	35	9	1.5	4	Many pulse movements 1 m.m.
1¼ "					4	Vagus stimulated; secondary at 150 m.m: marked slowing of heart and fall in blood-pressure, temporarily.
2 "					5	Vagus stimulated; secondary at 150 m.m: distinct slowing and slight fall in blood-pressure, temporarily.
5½ "	141	33	9	2.0	-	Many pulse movements 1.5 m.m.
9 "					6	Vagus stimulated; secondary at 150 m.m: marked slowing and fall in blood-pressure, temporarily.
11 "	110	34	10	2	-	Pulse movements about 1 m.m.
12 "						

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
After * 12 min.						0.01 gram per kilogram injected intravenously. (second injection.)
13 "	121	36	9	2	7	Pulse movements irregular in size. The largest are 1 m.m.
14 "						Vagus stimulated; secondary at 120 m.m: very slight effect
18 "	98	35	10	2	-	Pulse movements small and of irregular size.
19 "					8	Vagus stimulated; secondary at 100 m.m: very slight effects.
20½ "	108	38	12	1.5	-	
21 "						0.01 gram per kilogram injected intravenously (third injection).
22¼ "	117	39	11	1.5	9	Pulse movements small and of irregular size.
22½ "					9	Vagus stimulated; secondary at 80 m.m., very slight effects.
24 "						0.01 gram per kilogram injected intravenously (fourth injection).

After the fourth injection the blood-pressure fell steadily and no further record was taken. Five minutes after death the diaphragm responded to electrical/

rical stimulation of a phrenic nerve with the secondary coil at 400 m.m., and to stimulation of its muscle directly at 200 m.m.

In this experiment the general effects were not different from those in experiment XXXII. After the first injection stimulation of the vagus appeared to produce greater effects than the same strength of stimulus had elicited before injection: - Compare fig. 4 with fig. 2, and fig. 5 and 6 with fig. 1.

After the second and third injections and coincident with the diminished extent of the pulse and respiratory movements, stronger stimuli than those applied before any injection produced but slight effects on the rate of the pulse and the height of the blood-pressure - compare fig. 8 and 9 with fig. 2.

Experiment XXXIV. Weight of rabbit, 1750 grams. 0.002 gram of Sulphate of Atropine per kilogram was injected before extract of Homeria collina and this dose was repeated in 25 minutes. After the first dose of Sulphate of Atropine, 0.07 gram of extract of Homeria collina was injected into a jugular vein in four equal parts. Each portion was dissolved in 2 c.c. of Ringer's solution and was equivalent to 0.01 gram per kilogram; the total dose was equivalent to 0.04 gram per kilogram. The right vagus was cut and its peripheral end was stimulated from time to time. Plate XI.

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
Before *						
7 min.						Vagus stimulated; secondary at 130 m.m., marked slowing and fall in blood-pressure, temporarily.
6 "						Sulphate of Atropine, 0.002 gram per kilogram, injected intravenously.
1 "	88	39	6	3	-	Pulse movements 0.5 m.m.
½ "					1	Vagus stimulated; secondary at zero m.m. No effect.
*						0.01 gram of Extract per kilogram injected intravenously (first portion.)
After *						
1 min.	114	45	6	4	2	Pulse movements 0.5 m.m.
1½ "					2	Vagus stimulated; secondary at zero m.m. No effect.
3½/						

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Respirations per 10 seconds.	Respiration Excursus in m.m.	No. of Fig.	Notes.
After *						
3½ min.						Vagus stimulated; secondary at zero. No effect.
42/3 "	114	43	7	3.5	3	Pulse movements 0.5 m.m.
5 "					3	0.01 gram of Extract per kilogram injected intravenously (second portion).
6 "	131	50	7	4	-	Pulse movements less than 0.5 m.m.
7 "	113	52	7	3.5		
7½ "						Vagus stimulated; secondary at zero. No effect.
11½ "						Ditto.
15 "	100	52	8	3	-	Pulse movements less than 0.5 m.m.
16 "						Vagus stimulated; secondary at zero. Doubtful effect.
19 "						Sulphate of Atropine, 0.002 gram per kilogram, injected intravenously.
22 "						0.01 gram of Extract per kilogram injected intravenously (third portion).
28 "						0.01 gram of Extract per kilogram injected intravenously (fourth portion).
29					4	

After the third portion of extract the blood-pressure rose a little and remained at the higher level/

level it attained. After the fourth portion of extract the blood-pressure fell rapidly to zero. The respiratory movements ceased before those of the pulse did (fig.4), and no asphyxial convulsions occurred.

Although the Vagus was frequently stimulated with the secondary coil at zero m.m., from the time of administration of the second dose of Sulphate of Atropine (19 min. after *) till the blood-pressure fell finally, no effect on the pulse rate or the level of the blood-pressure resulted.

Throughout this experiment the Vagus was paralysed by Atropine. After each of the first three injections a rise in blood-pressure occurred and was maintained for a considerable time. After the fourth injection the blood-pressure fell rapidly and death occurred.

The pulse-rate was accelerated after the administration of Homeria collina; the pulse movements were not increased at any time.

The rate of the respirations was increased after Homeria, coincident with the increase in the rate of the pulse, and the extent of the respiratory movements became greater after the first two portions of the extract.

When the blood-pressure fell after the fourth injection, the extent of the respiratory excursus diminished rapidly, and respiratory movements ceased before the pulse movements.

These/

These three blood-pressure experiments show that extract of Homeria collina causes the following effects:- A rise in the blood-pressure occurs which is important both in its extent and in its duration. This rise takes place independently of the activity or the paralysis of the inhibitory terminations of the Vagus nerve.

The pulse rate is slowed but to an unimportant degree, and the extent of the pulse-movements is slightly increased. When the Vagus is paralysed by Atropine, the subsequent administration of Homeria collina causes an increase in the pulse rate, and no increase in the size of the movements.

From experiment XXXIII it would appear that, in the early stages of the action of the extract, electrical stimulation of the Vagus produces the usual effects on the pulse rate and blood-pressure in a greater degree than the same strength of stimulus did before the administration; while at a later stage comparatively strong stimulation of the Vagus produces but slight effects.

Paralysis of the inhibitory function of the Vagus nerve by Atropine neither prevents nor modifies the development of the more striking effects of Homeria collina in blood-pressure experiments.

Experiment/

Experiment XXXV. In the following experiment a cat, weighing 2800 grams, received into a jugular vein 0.028 gram of extract of Homeria collina in 2 c.c. of Ringer's solution. This is equivalent to a dose of 0.01 gram per kilogram. The blood-pressure and the volume of a loop of intestine were recorded. Plate XII.

Time.	Average blood-pressure in m.m. of Hg.	Pulse rate per 10 seconds.	Size of Pulse-movements.		Height of Plethysmo-gram above abscissa.	Notes.
			Carotid.	Intestine		
10 seconds before *	142	21	5 m.m.	1 to 1.5 m.m.	29 m.m.	See Plate.
*						0.01 gram of Extrac per kilogram inject ed intravenously. The blood-pressure fell a little, momentarily.
20 seconds after *	152	21	5 "	1 m.m. and under	25 "	See Plate.
30 sec. "	156	21	5 "	1 m.m. and under	25 "	Ditto.

The above analysis and the accompanying plate show that, after the injection of the extract, a rise in blood-pressure occurred simultaneously with a diminution in the intestinal volume and in the size of the intestinal pulse movements. At the same time the size of the carotid pulse movements, and the rate of the pulse were unchanged.

It/

It would appear, therefore, that contraction of the peripheral bloodvessels plays an important part in the rise of blood-pressure which follows the intravenous administration of extract of Homeria collina.

d. Lymph Hearts.

After the subcutaneous injection of lethal doses of the extract, the lymph hearts of the frog (Rana temporaria) cease contracting a considerable time before the final arrest of the blood heart occurs.

With frogs whose brains had been destroyed and whose blood heart and lymph hearts were exposed, experiments were performed to determine this action. It was found, for example, that after the subcutaneous injection into the left flank of a dose of extract equivalent to 0.5 gram per kilogram or about three times the minimum-lethal dose,, the lymph hearts were finally arrested in $2\frac{1}{2}$ hours and the blood heart continued beating, though feebly, for 30 minutes longer.

e. Blood.

The urine of rabbits poisoned with Homeria collina contained no blood corpuscles or haemoglobin, and post mortem examination did not reveal any signs of intravascular clotting. When blood was drawn from the rabbit's ear into capillary tubes from time to time after the injection of the extract, the serum which separated was not stained with haemoglobin. The coagulability of the blood does not appear to be altered.

F. Effects on Respiration.

In the investigation of the general effects on frogs it was observed that, after the administration of the extract, the respiratory movements became slowed and irregular in time, the flank respirations showing these effects earlier than the throat respirations, and that respiratory movements of the throat occurred when no cardiac impact could be detected.

Soon after the injection of Homeria collina in warm-blooded animals the respirations were slightly accelerated and became irregular in time. The period of acceleration was brief and, thereafter, the respiratory movements were slower and deeper. During this/

this period of slowed respiration it was observed in cats that expiration was slowed and inspiration was rapid. In rabbits, periods of inspiratory dyspnoea frequently occurred, accompanied by sounds apparently produced in the glottis. As death approached, the respirations became rapid and shallow, coincident with great irregularity in the force and rate of the cardiac impacts.

In the blood-pressure experiments on rabbits the rate of the respirations was diminished and the extent of the respiratory movements was increased in the earlier stages of the action of Homeria collina; in the later stages of poisoning, the extent of the respiratory movements was diminished, the rate increasing again. When the final fall in blood-pressure occurred the respirations became very slow and feeble. If single rapidly-fatal doses were given, respiratory convulsions accompanied the final fall in blood-pressure, but where death occurred less rapidly, as after several small doses of the extract, the respiratory movements ceased before the pulse movements. In these latter cases a few gasping respirations sometimes followed a long period of apnœa. Immediately after death a contraction of the diaphragm resulted from the application of weak electrical stimuli to a phrenic nerve or more powerful stimuli to the muscle directly.

It appears, therefore, that there may be a direct action on the respiratory centre, although the striking effects/

effects of the extract on the circulatory system produce secondary changes in the respirations.

G. Effects on the Temperature.

Apart from trifling variations in the rectal temperature of rabbits of less than 1°C., during experiments to determine the general action of Homeria collina the temperature of these animals was unaffected until it fell when the animal was moribund.

SUMMARY.

Homeria collina, Vent. - var. miniata is a native of South Africa and one of the Irideae. Cattle frequently die after eating the aerial parts of the growing plant. The corms of Homeria collina were used in this investigation, and from them two extracts were prepared, the one containing those substances which are soluble in ether, the other containing those substances which are soluble in 45% alcohol and being free from substances soluble in ether.

Neither the corms nor the extracts prepared from them appear to give distinctive colour reactions with chemical reagents. The alcoholic extract contains

a/

a glucoside which is probably the active principle. The ether extract is pharmacologically inert. The following is a precis of the pharmacology of the alcoholic extract:-

A. The minimum-lethal dose is almost the same for rabbits and cats; for frogs it is four times as great as for rabbits; rats are but slightly affected by fifty times the minimum-lethal dose for rabbits.

B. The most striking effects are those produced on the heart muscle. Gastro-intestinal irritation is not produced. The activity of the extract is not destroyed by a considerable amount of heat.

C. The extract increases, and later abolishes, the reflex function of the spinal cord by a direct action.

The terminations of sensory nerves are not affected by the extract, but the function of the motor nerves is impaired and then abolished, owing to an action on the nerve endings in muscle.

D. In contact with the extract, skeletal muscle shortens; stronger stimuli are required to cause a contraction, and finally the muscle becomes inexcitable, rigid and acid in reaction.

Old solutions of extract cause fibrillary twitches to appear in the poisoned muscle.

E. The rate of the heart's contractions is slowed, the/

the size of the diastolic and systolic movements of the auricles and ventricle is increased, and the diastolic pause is prolonged. Smaller quantities of the extract cause the heart to be arrested in diastole, after which it slowly passes into permanent systole. Larger amounts of extract arrest the heart in systole. Soon after its final arrest the heart muscle becomes acid in reaction.

Paralysis of the vagus nerve by Atropine does not prevent or modify the important effects of Homeria collina upon the heart muscle.

The cardio-inhibitory effect of vagus stimulation is increased in the early stages of poisoning but diminishes in the later stages.

The extract causes the blood-vessels to contract, but this action is not a powerful one.

The blood-pressure rises after the administration of the extract and remains high for a considerable time.

The lymph hearts are arrested before the blood-heart.

There is no evidence that haemolysis occurs in vivo or that the coagulability of the blood is affected.

F. Many of the effects on the respiration result from/

from the action of Homeria collina on the heart, but there is probably a direct central action on respiration.

G. The administration of this substance does not affect the temperature of the body.

Homeria collina is, therefore, a new addition to that group of plants whose active principles produce their most striking effects on the heart muscle, - the group which embraces Digitalis, Strophanthus, Scilla maritima and Convallaria majalis among others.

Conjecture as to the therapeutic value of Homeria is worthless; nothing short of the actual administration in suitable cases can decide whether it is worthy to rank with Digitalis and Strophanthus.

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